MYOFASCIAL TRIGGER POINTS AND INNERVATION ZONE LOCATIONS IN UPPER TRAPEZIUS MUSCLES

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ABSTRACT

Myofascial pain syndrome is characterized by sensory, motor and autonomic symptoms, and a myofascial trigger point (MTrP) is considered the principal clinical feature. Clinicians recognise myofascial pain syndrome as an important clinical entity but many basic and clinical issues need further research. Electrophysiological studies indicate that abnormal electrical activity is detectable near MTrPs. This phenomenon has been described as endplate noise and it has been purported to be associated MTrP pathophysiology. Authors also suggest that MTrPs are located in the innervation zone (IZ) of muscles. The aim of this thesis was to describe both the location of MTrP and the IZ' locations in the upper trapezius muscle. The hypothesis was that distance between the IZ and the MTrP in upper trapezius muscle is equal to zero.

This thesis includes two preliminary studies. The first one address the reliability of surface electromyography (EMG) in locating the IZ in upper trapezius muscle, and the second one address the reliability of a manual palpation protocol in locating the MTrP in upper trapezius muscle. The intra-rater reliability of surface EMG in locating the IZ was almost perfect; with Kappa = 0.90 for operator A and Kappa = 0.92 for operator B. Also the inter-rater reliability showed an almost perfect agreement, with Kappa = 0.82. Both the operators conducted 900 estimations of IZ' location through visual analysis of the EMG signals. The reliability of an experienced physiotherapist using a manual palpation protocol in locating the MTrP in the upper trapezius was established. An anatomical landmark system was defined and MTrP' location established using X and Y values. The ICC values were 0.62 for X and 0.81 for Y. Twenty-four subjects with MTrP in upper trapezius were enrolled for this latter study.

MTrP' and IZ' locations were described in 48 subjects. MTrPs were located in well-defined areas of the upper trapezius, showing a typical location with a mean distance from the IZ of 10.4 ± 5.8 mm. MTrPs in the upper trapezius are proximally located to the IZ but not overlapped by it ($p = 0.6$). These results extend the body of knowledge regarding the phenomenon of MTrP iperalgesia.

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TABLES OF CONTENS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

PUBLICATIONS ARISING FORM THIS THESIS

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ABBREVIATIONS

AA	Acriomial angle
ACh	Acetylcholine
AChE	Enzyme acetylcholinesterase
AChR	Acetylcholine receptor
A/D	Aanalog-to-digital
ALS_d	Distance between C7 and AA
ALS	Anatomical landmark system
BMI	Body mass index
CNP	Chronic neck pain
C7	Spinous process of the seventh vertebrae
EMG	Electromyography
EPN	End plate noise
GPRD	General Research Database
H_0	Null hypothesis
ICC	Intraclass correlation coefficient
IED	Interelectrode distance
IZ	Innervation zone
MHRA	Medicines and Healthcare products Regulatory Agency
MPS	Myofascial pain syndrome
MTrP	Myofascial trigger point
MTrP_1	MTrP detected during the first palpatory examination
MTrP_2	MTrP detected during the second palpatory examination
MVC	Maximal voluntary contraction
NDI	Neck disability index
NIH	National Institutes of Health
NIHR	National Institute for Health Research
Ph	Power of hydrogen
PPT	Pain pressure threshold
QALY	Quality-adjusted life years
SD	Single differential
SEA	Spontaneous EMG activity
sEMG	Surface electromyography

VAS	Visual analogic scale

CHAPTER 1

GENERAL INTRODUCTION TO MYOFASCIAL TRIGGER POINTS

1.1 MUSCULOSKELETAL PAIN: THE WIDER PERSPECTIVE.

Pain is a widespread negative experience involving different ages, races and cultures. It affects all individuals in their life and despite its common occurrence, it is difficult to understand, explain and treat. A simple, Cartesian model would postulate a direct link between the amount of pain experienced and the amount of pathology incurred by the tissues from which that pain emanates or which pain is referred to (Goldberg, 2008). However, research has shown that there isn't always a direct relationship between the degree of tissue pathology and the pain experience, and modern medicine has replaced the old Cartesian model with more complex models (Goldberg, 2008).

Musculoskeletal pain is a major medical problem and the related disorders are the leading cause of long-term disability in modern societies (Brooks, 2006, Woolf and Pfleger, 2003). Even if they are not life-threatening, they significantly affect the psychosocial status of the patients as well as their quality of life (Salaffi et al., 2005, Woolf and Akesson, 2001).

Data from the General Research Database (GPRD), jointly funded by the NHS National Institute for Health Research (NIHR) and the Medicines and Healthcare Products Regulatory Agency (MHRA), reports that in 2001 the incidence of musculoskeletal conditions in the general population was 947 per 10000. The data set was based on attendance in general practice and revealed that the largest group was tissue rheumatism and chronic widespread pain (figure 1.1). An additional data set from the same database, collected ten years previously, clearly shows a trend toward the increase in prevalence of musculoskeletal conditions, especially in subjects aged over 45 (figure 1.2). Almost one third of people aged over 75 reports a musculoskeletal problem (Urwin et al., 1998) and the overall prevalence of musculoskeletal conditions rises in the aged population (Jordan et al., 2007). Notably, different general practice consultation databases report a higher female prevalence for musculoskeletal conditions with a ratio of 1:1.3 (figure 1.3).

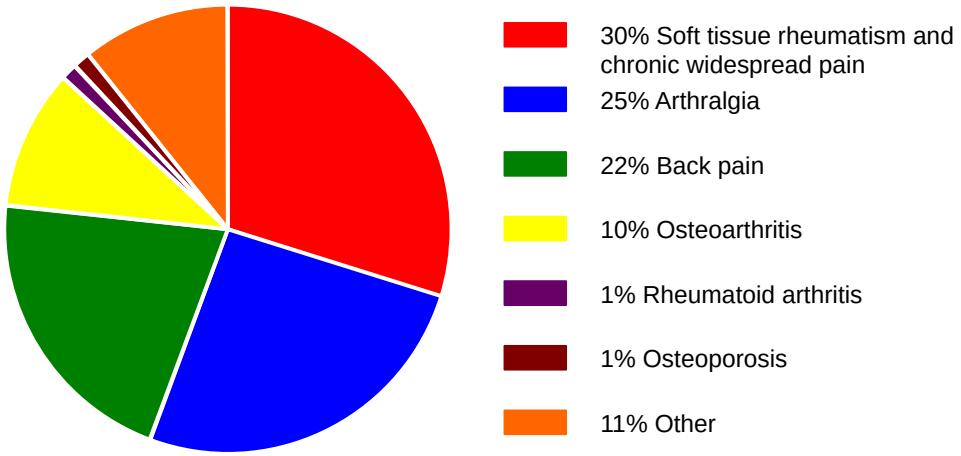

Figure 1.1: Epidemiological data for musculoskeletal pain from the General Practice Research Database (adapted from Parsons & Symmons, 2010).

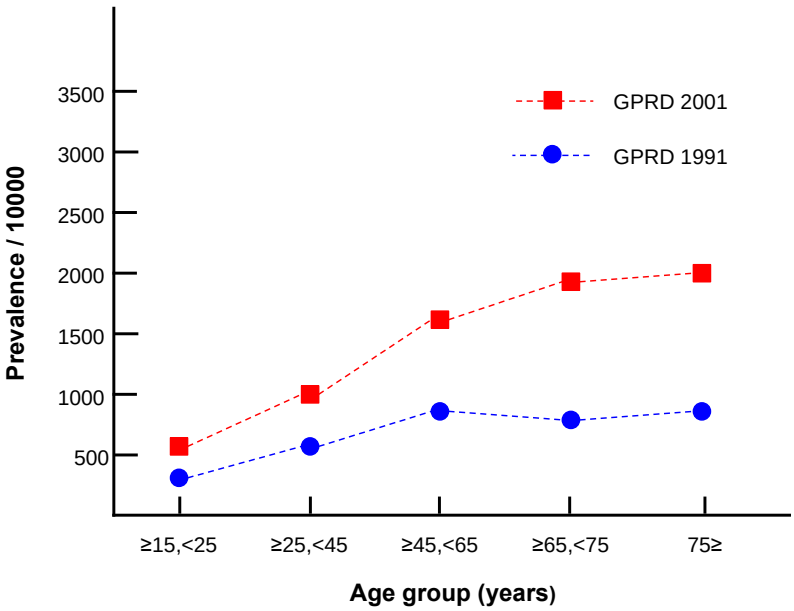

Figure 1.2: Prevalence of musculoskeletal pain in different aged groups.

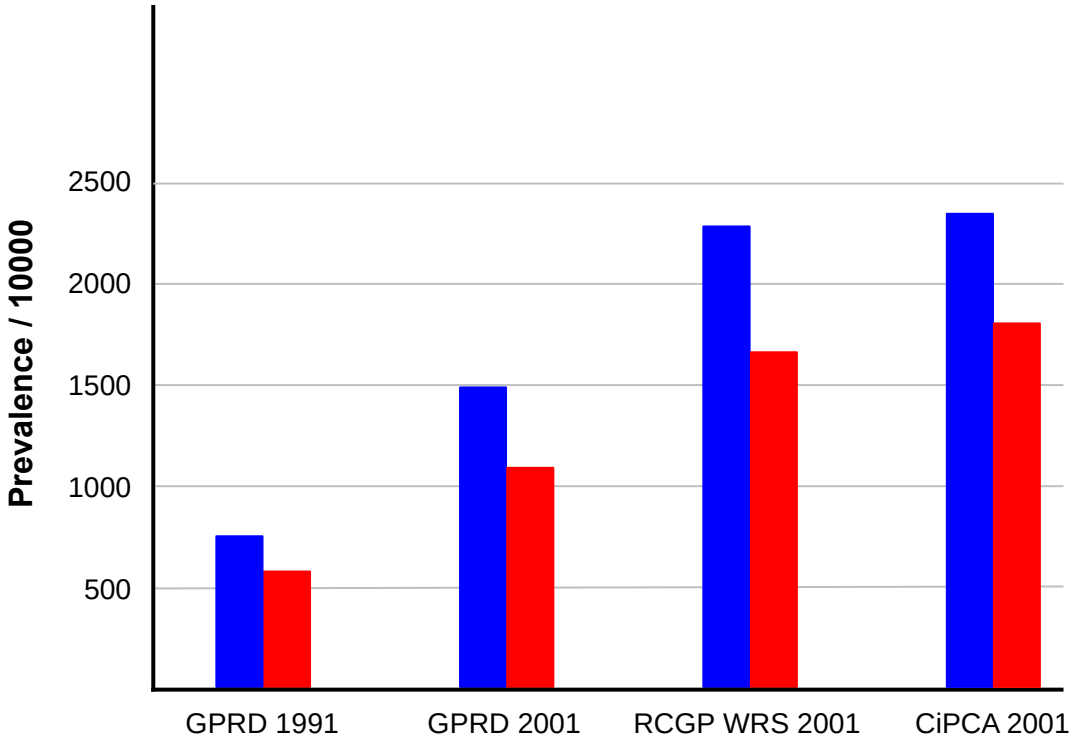

Figure 1.3: Sex prevalence rates of musculoskeletal conditions from different consultation databases per 10000 people aged >15 years.

A Japanese study projected the national burden of back, hip, and knee pain from 2005 to 2055 in terms of quality-adjusted life years (QALY). It has been estimated that losses of QALY associated with back, hip, and knee pain per 1000 population will increase from 17.2, 3.8, and 8.9, respectively in 2005 to 18.8, 4.5, and 11.2, respectively by 2055 (Suka and Yoshida, 2009).

Due to the population rising and aging, research prevention and treatment of musculoskeletal disorders is considered to be one of the healthcare priorities (Woolf and Pfleger, 2003). Musculoskeletal pain is a consequence of repetitive strain, overuse and micro or macrotrauma (Petty and Moore, 2001). It can be acute or chronic, focal or diffuse and many different words are used to describe various aspects of the pain experiences. It is estimated that there are more than 200 types of musculoskeletal disease (Parsons and Symmons,

2010), and for most of them, pain is the main complaint. They include various types of degenerative joint and soft tissue disorders, osteoporosis, arthritis and systemic connective tissue diseases (Parsons and Symmons, 2010).

In 2001, Mense proposed to classify these clinical disorders into two main groups, articular and nonarticluar (Mense et al., 2001). Articular disorders include joint diseases involving inflammation, varying degrees of trauma or degenerative process of the synovial joints. Two examples with a high epidemiological impact are osteoarthritis and rheumatoid arthritis. Osteoarthritis is uncommon among young subjects but in the elderly the prevalence approaches 70% (Cicuttini and Spector, 1995) while rheumatoid arthritis occurs in 1% of the general population (Parsons and Symmons, 2010). Articular group also includes joint dysfunction usually characterized by mechanical pain arising from conditions such as hypomobility, hypermobility and instability. Conversely, nonarticular disorders affect periarticular tissue such as fascia, muscles, tendons, bursae, and nerves. Typical examples include plantar heel pain resulting from a non-inflammatory degenerative process of the plantar fascia (Lemont et al., 2003), achilles tendonitis (Enwemeka, 1989), myofascial pain syndromes (MPS) (Giamberardino et al., 2011), and fibromyalgia (Jafri, 2014, Chinn et al., 2016).

The classification described above can give an insight into structures involved in nociception. It supports clinicians in their decision making both during the assessment and the treatment selection. Diagnostic tests or palpation procedures can be selected considering their diagnostic properties as well their capacity to induce a mechanical stress in a specific anatomical structure. With a similar approach, interventions should be selected by considering their underlying mechanism and the target tissue. Pain is a complex neurophysiological process where nociception from somatic structures is only part of the pain experience. Advances in pain science underlined the importance of other mechanisms involved in the pain elicitation (Gifford and Butler, 1997, Jones, 1995). Central sensitisation and peripheral neuropathic are two examples of mechanisms responsible for pain generation and/or maintenance (Smart et al., 2010, Smart et al., 2011, Smart et al., 2012a,

Smart et al., 2012b, Smart et al., 2012c). Patients with central sensitization show pain hypersensitivity tactile allodynia, pressure hyperalgesia, and aftersensations. Central sensitization develops in the presence of long-lasting nociceptor inputs that increase the excitability and synaptic efficacy of neurons in central nociceptive pathways (Woolf, 2011). Peripheral sensitization is defined as a reduction in threshold and an amplification in the responsiveness of nociceptors (Latremoliere and Woolf, 2009). It occurs when, due to a tissue damage, primary sensory neurons are exposed to inflammatory mediators (Chen et al., 1999, Petho et al., 2001). Consequently, peripheral sensitization is restricted to the site of tissue injury while central sensitization is widespread phenomenon (Hucho and Levine, 2007). A growing body of experimental data has provided some evidence suggesting that central sensitisation may underline some commonly encountered clinical presentations of musculoskeletal pain including low back pain, neck pain, whiplash and MPS (Nijs et al., 2010, Woolf, 2011, Srbely et al., 2010). The diagnostic classification of patients with pain symptomatology could be challenging for clinicians. Widespread pain, long lasting pain, allodynia, hyperalgesia, and pain in the presence of cognitive or affective dysfunctions can be potentially associated with all the musculoskeletal disorders (Butler and Matheson, 2000, Nijs et al., 2010, Woolf, 2011).

The need for a pain mechanisms-based approach has been suggested for both clinicians and researchers (Jones, 1995, Gifford and Butler, 1997) as it can turn into more appropriate evaluation of patients affected by musculoskeletal disorders. It is important to understand how subjects affected by the same pathology, may have a different presentation of pain symptoms. It can be local or widespread, moderate to intense, mechanical or spontaneous, aching or sharp. Smart and collaborators (2012) for example, with the aim of improving low back pain treatment, completed a series of studies to help clinicians to recognize different pain patterns in subjects with low back pain (Smart et al., 2012a, Smart et al., 2012b, Smart et al., 2012c).

Future research should be aimed at improving the clinical evaluation of musculoskeletal pain, especially in cases of patients complaining of

widespread pain. It constitutes a major health issue and it is a big subgroup of the population with musculoskeletal pain. Widespread pain' prevalence in Korean communities has been estimated around 12% (figure 1.4) (Cho et al., 2012), which is essentially the same rate observed in Caucasians (Croft et al., 1993). Considering the gender' difference, widespread pain in Koreans was respectively 5.5% in males and 16.2% in females, and for females, it rises to 20% after the age of 60.

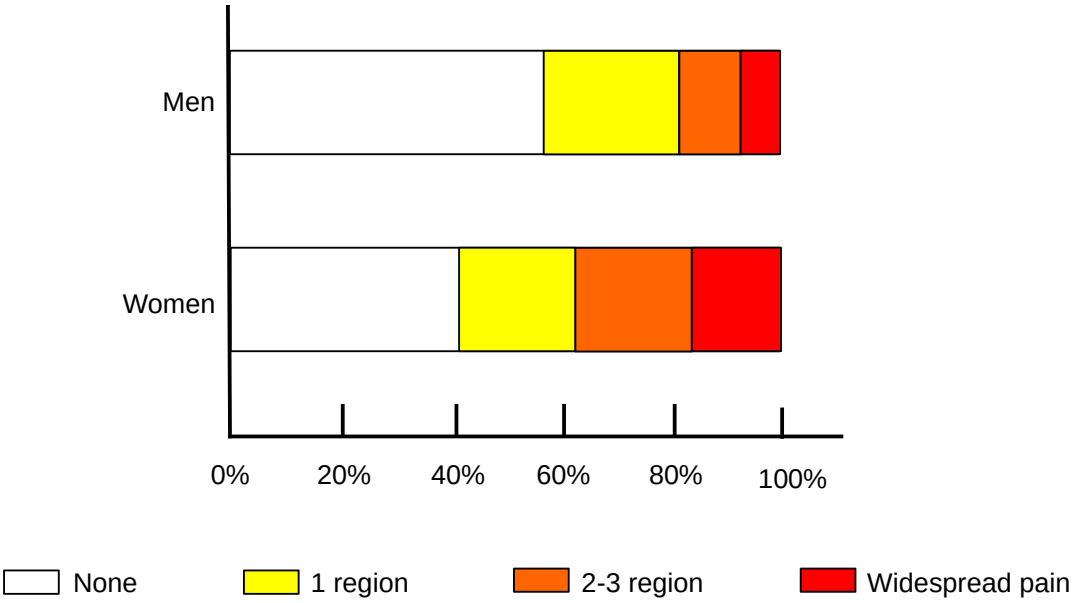

Figure 1.4: Prevalence of musculoskeletal pain according to the number of painful regions (Adapted from Cho et al., 2012).

A recent cross-sectional study, investigating the prevalence and the characteristics of multisite musculoskeletal pain, provided a detailed view on widespread pain among 3740 French workers. The extent of multisite pain during the past 12 months were noteworthy, 83.8% for males and 83.9% for females. The prevalence of multisite pain affecting more the one sites was 3 to 12 times more frequent than prevalence of pain at only one site (figure 1.5

and 1.6). When considering musculoskeletal pain lasting at least 30 days, the prevalence dropped to 32.8% for males and 37.3% for females, and two-thirds complained of pain in more than one sites (Parot-Schinkel et al., 2012).

Some questions that can be asked are: what are the clinical profiles and the determinants in these patients with widespread pain? Is it possible to ascribe them a specific diagnosis? If we exclude studies centred on fibromyalgia or other rheumatic disorders, there is paucity of epidemiological data aimed to provide a diagnosis for the patient with widespread or multisite pain.

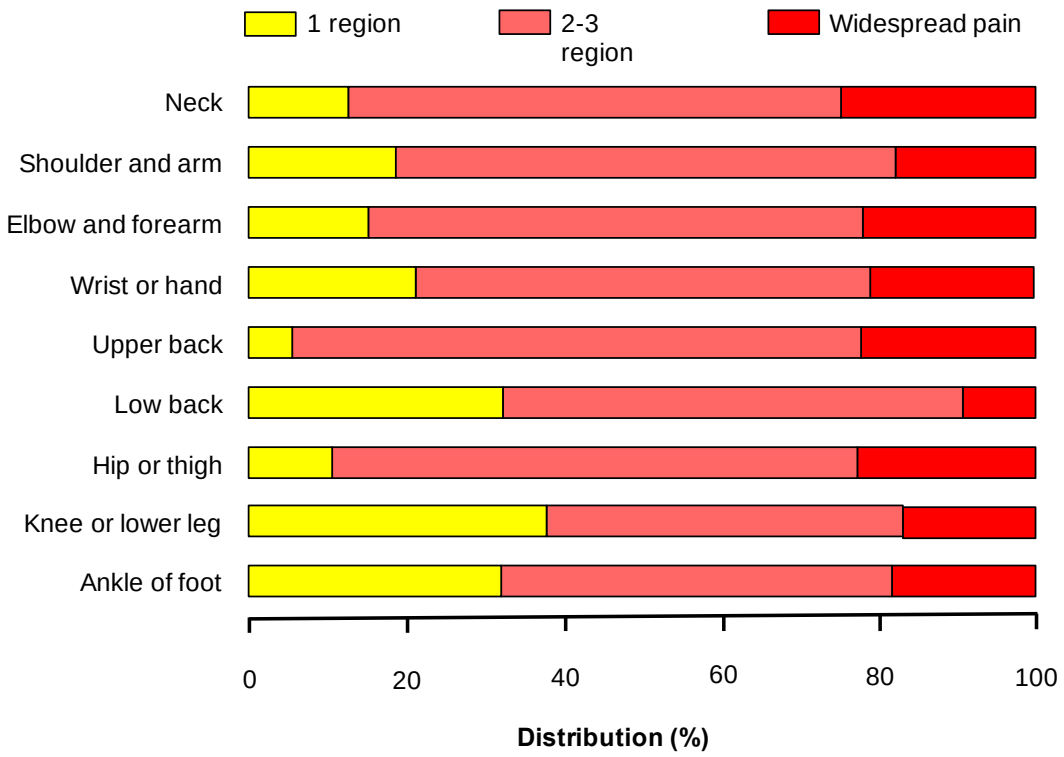

Figure 1.5: Distribution of multisite pain lasting at least 30 days among a selected population of male French workers (Adapted form Parot-Schinkel et al., 2012).

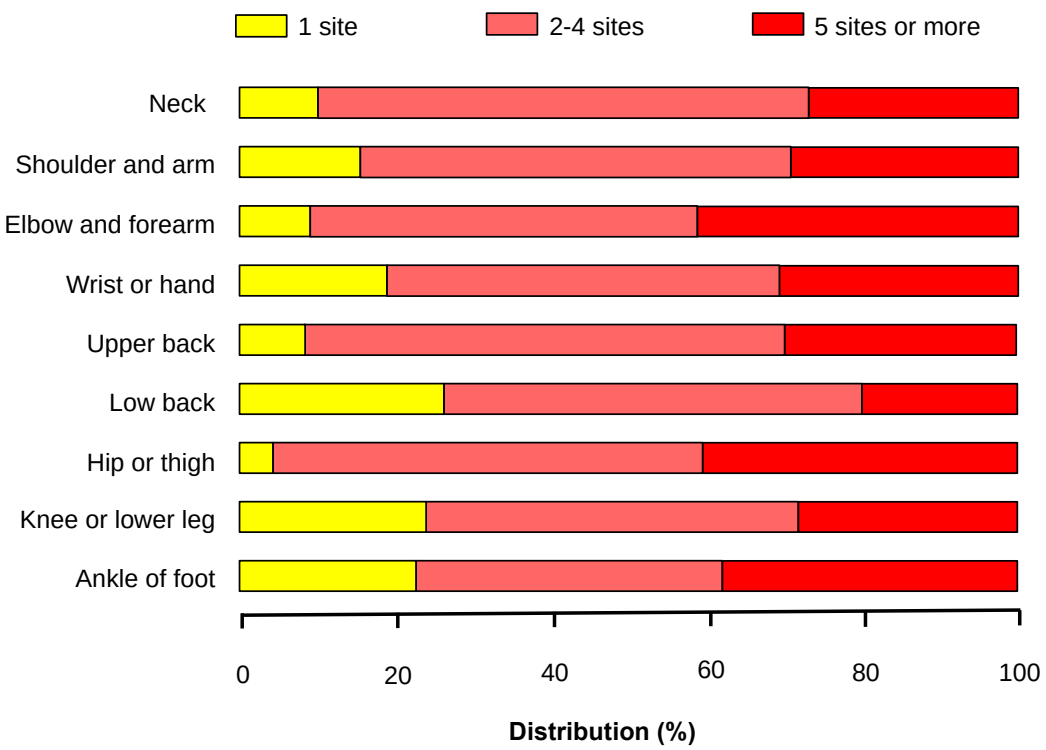

Figure 1.6: Distribution of multisite pain lasting at least 30 days among a selected population of female French workers (Adapted form Parot-Schinkel et al., 2012).

1.2 DEFINITION OF MYOFASCIAL PAIN SYNDROME AND MYOFASCIAL TRIGGER POINTS

The original and the most commonly accepted definition of MPS has been formulated by Travell and Simons (1983) in the first volume of *The Trigger point Manual* edited in 1983. The authors defined the MPS as a regional pain characterized by the presence of one or more active myofascial trigger points (MTrPs). They recommend that it should be considered a specific diagnosis and clinicians should avoid using this term to refer to soft tissue pain in general or in other circumstances where a proper diagnostic category is not identified. An active MTrP is a distinctive clinical characteristic of this painful syndrome and it is defined as a hyperirritable palpable nodule contained in the skeletal muscle fibres. It can produce referred pain, either on digital compression or spontaneously (figure 1.7). If stimulated with dry needling or snapping palpation, it may exhibit a typical muscle fasciculation or jump sign (a typical apprehension reaction to painful stimulus) (Simons, 1996).

MTrPs can be classified as active or latent. A latent MTrP does not cause spontaneous pain but may restrict movement (Grieve et al., 2011, Trampas et al., 2010, Aguilera et al., 2009) or cause muscle weakness (Ge et al., 2012). Pain in this case only occurs with the application of vigorous digital pressure (Simons, 1996). Pain pressure threshold of latent MTrPs have been described only in a few clinical studies. For instance, Gemmell and Hilland (2011) (Gemmell and Hilland, 2011) reported a mean PPT value of 4 kg/cm^2 for latent MTrPs in upper trapezius muscles, and Xu et al. (2010) reported a mean PPT values of 450 KPa for latent MTrP in the tibialis anterior muscles. The clinical relevance of latent MTrP has never been investigated deeply although a review published in 2013 on Current Pain Headache Reports stated that its treatment is important to prevent its “activation” (Celik and Mutlu, 2013).

Conversely, an active trigger point is frequently responsible for the presenting complaint. Its PPT is lower than in latent MTrPs. For example, Llamas-Ramos et al. (2014) reported a mean PPT value of 188.1 KPa (i.e 1.9 kg/cm2) for

active MTrPs in upper trapezius muscles. With an active trigger point, manual palpation reproduces the patients' pain symptoms and in some case also autonomic phenomena like skin redness (vasomotor response), sweating (sudomotor response), goose pimpling (pilomotor response), lacrimation and dizziness (Travell and Simons, 1983). These possible reactions illustrate the dynamic nature of active MTrPs which can be limited to local muscle pain or can include complex referred pain (Mense and Gerwin, 2010). It is clear that "trigger" is an appropriate term to describe the clinical event of pain projection due to muscle palpation (referred pain). Especially in these cases, the digital compression stimulates pain at a location away from the stimulated site. It is important to note however, that a systematic investigation on MTrP symptomatology has never been performed.

To further support clinicians, Travell and Simons published two seminal textbooks, one in 1983 for the upper extremities and one in 1992 for the lower extremities (Travell and Simons, 1983, Travell and Simons, 1992). In 1992, the textbook on the lower extremities was updated and a second edition is available (Simons et al., 1999). The textbooks include different body charts for several skeletal muscles. Common MTrPs locations and their referral zones are described for each muscle. The pain patterns illustrations were based on a previous experimental study, conducted by Janet Travell and Seymour Rinzler, including 32 muscles and a population of 1000 subjects diagnosed with MPS (Travell and Rinzler, 1952). The results of the study were published in a classic contribution to the field entitled "The Myofascial Genesis of Pain" (Travell and Rinzler, 1952).

Over the past few decades, MPS have received greater attention in the scientific and clinical literature, and a survey by American pain specialists has revealed a general agreement that MPS is a legitimate medical diagnosis (Harden et al., 2000). Since 2005, the International Association for the Study of Pain has included MPS in the Core Curriculum for the Professional Education in Pain (Charlton, 2005).

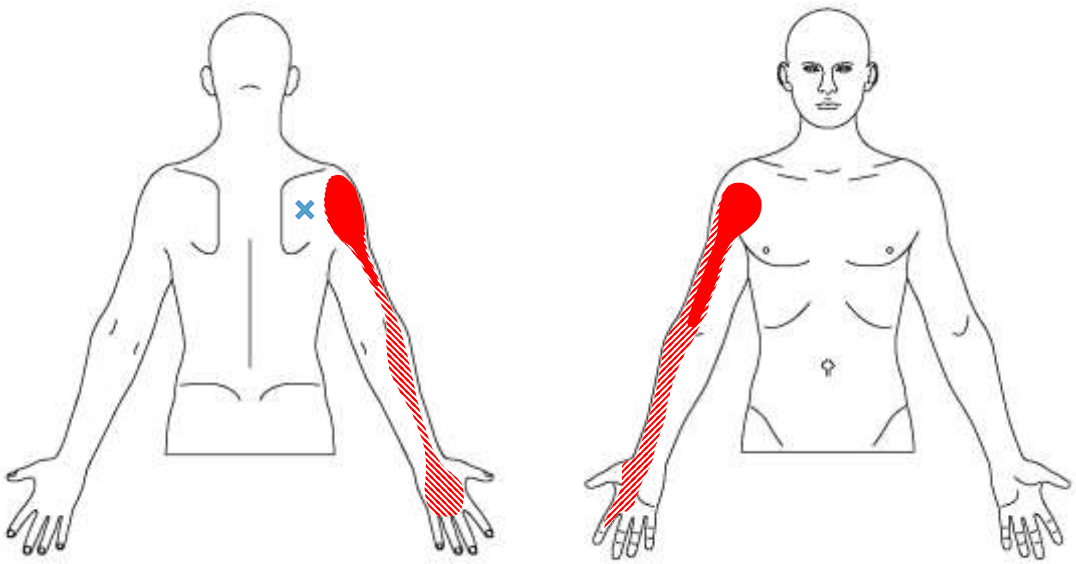

Figure 1.7: Trigger point in the infraspinatus muscle refers pain to the ipsilateral arm, forearm and hand. The blue cross indicate the approximate location of a possible the trigger point while the red areas show the referred pain extent (Adapted from Travell and Simons, 1983).

For effective management of patients with musculoskeletal disorders clinicians should be familiar with the clinical manifestations of MPS. Patients may complain of both an acute and chronic MPS. In both cases, the reported symptomatology is essentially muscle pain and it is described, as other somatic and visceral pain, like dull, aching and poorly localised (Mense, 2008, Bogduk, 2009). It is often reported as deep and when referred, it can mimic other pains such as radicular pain (Reynolds, 1981). It may occasionally be associated with a sensory component of paraesthesia (i.e. tingling, burning, prickling, tickling) or dysesthesia (i.e. abnormal sense of touch) (Mense et al., 2001). MPS diagnosis can be challenging for clinicians especially because pain may be felt elsewhere than where it originates. Moreover, it can persist long after the initiating cause has resolved, similarly to whiplash injuries or chronic low back pain. In this case, additional comorbidities are frequently reported and the patient's management becomes further complex. In such a complex clinical presentation, the MPS diagnosis is often underestimated

leading to frequent mistakes in diagnosis and in treatment of patients (Fricton, 1994, Gerwin et al., 2004, Suleiman and Johnston, 2001).

To assist practitioners in diagnosis of MPS, Simons (2004) remarked about the importance of accounting for the following history findings:

- Regional pain
- Onset with sudden muscle overload
- Onset with sustained muscular contraction in shortened position
- Onset with repetitive activity

MTrPs should be considered the main distinguishing features of MPS, and clinicians are required to develop effective manual skills to palpate muscle and detect their presence. A MTrP shows an abnormal muscle structure (i.e the taut band) and a localised hyperalgesia. Moreover, MTrPs may exert influences upon motor function; typically, a reduction of range of motion (Grieve et al., 2011, Grieve et al., 2013b) but also muscle inhibition or muscle imbalances (Lucas et al., 2010a, Roach et al., 2013). The taut band is a linear band of hardened muscle. It does not involve the entire muscle but just a limited numbers of fibres. Indeed, a muscle affected by a MTrP has a heterogeneous feel of hard and soft areas, rather than a homogeneous consistency (Mense and Gerwin, 2010). It is currently suggested that the taut band is composed of a limited number of contracted fibres that include some constantly shortened sarcomeres, thought to be located in the vicinity of the motor endplate zone (Mense, 2008). This localized muscle contraction is associated with the sensory phenomenon of spot tenderness. A gentle manual palpation along the taut band presents the opportunity to define the exact location of the spot tenderness. When pressure is applied on the spot tenderness, patients often recognize the pain as a familiar symptom and in the case of a hyper-irritable spot, react with an instinctive movement or exclamation. Simons named this apprehension sign “Jump sign” and suggested that it depends on the degree of MTrP irritability (Simons, 1996), but obviously depends also on the amount of pressure exerted by the examiner (Travell and Simons, 1983). Another distinguishing feature of MTrP

is the referred pain that is usually elicited by specific patient activities or postures. Alternatively, the referred pain can be induced by stimulating the spot tenderness with manual compression or dry needling. It usually spreads into a wide area adjacent or at distance from the MTrP' location, where original nociceptions are generated. Clinically, it is important to remember that the referred pain pattern is not dermatomal (Bogduk, 2009). Patients usually find it difficult to describe the boundaries of this area, but can define its centre. Interpretation of the referred pain can make the diagnostic process difficult, even if each muscle has its own specific pain pattern and those are quite consistent amongst subjects. MTrP charts including the pain maps, are a valid support during the history taking.

A specific motor sign associated with MTrPs is the local twitch response; once this is evoked by the mechanical stimulation of the spot tenderness. It is a transient contraction of some fibres that probably includes the taut band and it is clearly visible on the skin. According to an experiment conducted with patients affected by cervical radiculopathy, this transmission depends on the central nervous system, with a possible minor degree of local transmission (Hong, 1994b). Hong investigated the twitch response using EMG and defined it as high-amplitude, polyphasic electrical discharge. Clinicians elicit the local twitch response both in active and latent MTrPs by snapping palpation (i.e plucking of the muscle fascicle, like plucking of a violin) or dry needling (Hong and Simons, 1998). A schematic chart of MTrP and its clinical features, is illustrated in figure 1.14.

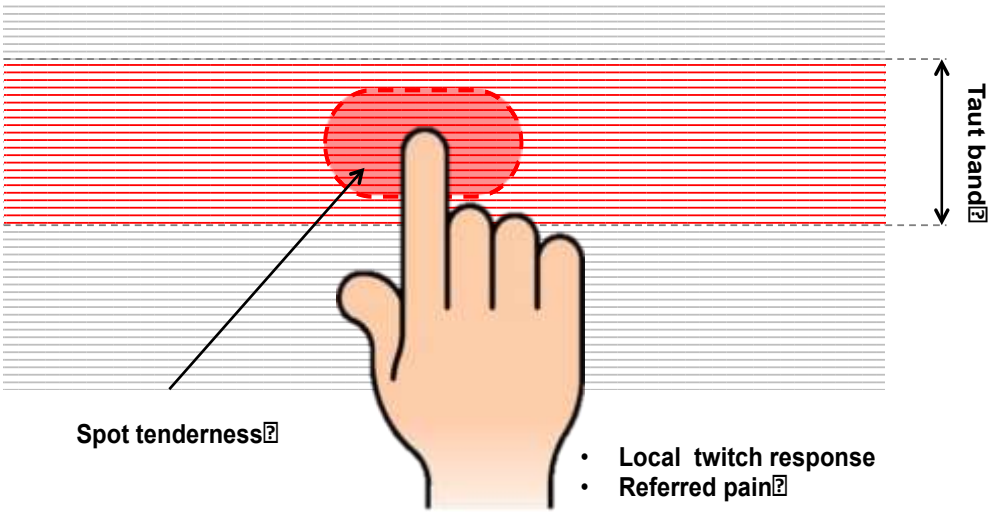

Figure 1.14: MTrPs' clinical features. MTrP is characterized by the presence of a taut band including a spot tenderness. Additionally, manual palpation can elicit additional signs as the referred pain and the local twitch response.

1.3 ADDITIONAL CLINICAL FEATURES

Beside the main clinical features, patients with MPS may complain of some additional symptoms and signs. The most commonly observed are: muscle weakness, reduced range of motion, altered muscle recruitment, and autonomic signs. They should not be considered specific for MPS but more as correlated motor impairments. Indeed, they are frequently observed in musculoskeletal disorders with different underlying aetiologies. Clinicians should be aware of these impairments during both the examination and treatment.

1.3.1 Muscle Weakness

Patients affected by MPS can exhibit weakness in specific active tasks. During muscle strength testing, muscles harbouring active MTrPs frequently show a relevant loss of maximal strength or a reduced resistance during submaximal contraction. The loss of strength is often evident when involved and uninvolved muscles are compared (Simons, 1996). Additionally, Celik and Yeldan showed in a group of 50 healthy adults, that even latent MTrPs in shoulder muscles could decrease muscle strength (Celik and Yeldan, 2011).

1.3.2 Range of motion

Muscles with MTrPs exhibit a limited extensibility and consequently, the involved joints may show a limited range of motion (Travell and Simons, 1983). A recent study demonstrated this in patients with acute whiplash associated disorders (Fernandez-Perez et al., 2012). The cervical range of motion showed a greater reduction in patients with a greater number of MTrPs and a lower pressure pain threshold over the neck (Fernandez-Perez et al., 2012). It would seem reasonable to expect that when a muscle with an active MTrP is stretched, pain is produced because the sensitized fibres of the taut band increase their tension. Also, two published case reports described patients with shoulder conditions and reduction of the shoulder range of motion (Passigli et al., 2016, Clewley et al., 2014). In both the cases, the MTrPs of the shoulder girdle (i.e. infraspinatus, teres minor, posterior deltoid) were treated using dry needling and the shoulder mobility improved. Restriction of the range of motion in the ankle has also been demonstrated in heathy subjects, with MTrPs in the triceps surae and soleus muscle (Grieve et al., 2011, Grieve et al., 2013b).

The reduction of motion due to MTrP is muscle specific and it varies from muscle to muscle. For example, the degree of movement limitation induced by MTrPs may be marked in the case of subscapularis muscle (Jankovic and van Zundert, 2006, Gupta and Singh, 2016, Shin et al., 2014). Conversely, the movement limitation may be difficult to be detected in a muscle like latissimus dorsi in which the muscle extensibility does not clearly affect its joint mobility (i.e. the shoulder). In such a case, electronic inclinometers can be used to accurately establish pre and post treatment improvements in patients with MPS (Shin et al., 2012, Brosseau et al., 1997).

1.3.3 Altered muscle recruitment

Alteration of muscle activation patterns occur due to pain in patients with neck and low back pain. Some muscles may show an inhibition and others on the contrary, a hyper-activation (Richardson et al., 2004). It is hypothesized by authors that similar motor control alterations can be associated with MTrPs.

Lucas and his collaborators investigated muscle activation patterns in scapular muscles harbouring latent MTrPs. They concluded that the presence of latent MTrP in upward scapular rotators (upper trapezius muscles, lower trapezius muscle and serratus anterior muscle) modifies the muscle activation pattern during scapular elevation (Lucas et al., 2010a), potentially increasing the risk of developing conditions like for example, rotator cuff injury, impingement syndromes and shoulder instability.

Moreover, the experimental muscle pain induced by injection of hypertonic saline, that can be considered a nociception similar to MTrP, has been found to evoke a reorganization of the spatial distribution of muscle activity and prevents the adaptation of the muscle activity during fatigue (Falla et al., 2010, Madeleine et al., 2006, Falla et al., 2009). This phenomenon has been described also in women with fibromyalgia (Falla et al., 2010). It is reasonable to suppose that MTrPs will induce similar changes and this scenario fits with the early manifestation of fatigue reported by patients with MPS. In support of this Ge and his collaborators showed in an EMG study, that latent MTrPs are associated with an accelerated development of muscle fatigue (Ge et al., 2012).

1.3.4 Autonomic component

Disturbances of the autonomic functions can be present in patients with MPS and include vasoconstriction, vasodilatation, lacrimation (in case of MTrPs in the orofacial region), and goosebumps (i.e pilomotor activity) (Travell and Simons, 1983). In musculoskeletal rehabilitation, these dysfunctional phenomena are often un-noted, although they are very important for the patient management. A systematic examination of patients with MPS is recommended to establish the degree of autonomic dysfunction and to avoid adverse effects during treatments. Vasoconstriction can be tested with the back of the examiner's hand; the affected area will be cooler if compared with adjacent areas. Conversely, in the of case of vasodilatation, a pattern of sweating will be noted. A pilomotor reflex can be observed when the involved area is stimulated with manual palpation or dry needling.

1.4 A BRIEF HISTORICAL REVIEW.

The current understanding of MPS and the related MTrP has been developed progressively during the past century. MTrPs are a common cause of muscular pain and authors from different countries have identified and described them innumerable times. They have been identified using different names, focusing mostly on the specific anatomical area and thus providing a limited interpretation of this clinical phenomenon (Travell and Simons, 1983).

It was 1841 when Francoise Valleix, in his "*Traité des Neuralgies; ou, affections douloureuses des nerfs*" (Essay on neuropathic pain and nerve disorders) for the first time, carefully described "l*es points douloureux*"(the painful spots) that emanate pain due to digital pressure (Valleix, 1841). He stated: "*If, in the interval of the shooting pains, one asks a patient what is the seat of his pain, he replies then by designating limited points. It is only with the aid of pressure that one discovers exactly the extent of the painful points. They are found placed in four principal points of the trajectory of different nerves*" (Valleix, 1841). This started a debate, which persisted for many years, as to whether pain arises from muscles or nerves. Walleix pointed out "… *if the pain spreads into the muscles, the muscular contractions are principally painful. This is muscular rheumatism*" (Valleix, 1841).

The German physician Cornelius, in 1903, contributed to this hypothesis asserting that the nerve endings at these points, which he called "Nervenpunkte" (Neural point), are sensitized as a consequence of both physical and emotional stress (Cornelius, 1903).

In the following years, other German authors again identified clinical phenomena characteristic of the MPS, as a muscular rheumatism (Llewellyn and Jones, 1915, Schmidt, 1918). Additionally, in 1931, Lange authored the first trigger point manual using the term "myogelosis", where the term clearly referred to the palpation findings (Lange, 1931).

Hunter, a Canadian physician, in 1933 reported a few cases in which abdominal pain arose from points of tenderness in the abdominal muscles (Hunter, 1933). Similarly in 1936, Edeiken and Wolferth, at the University of Pennsylvania Medical School, noticed that patients with coronary thrombosis could develop shoulder pain that had been elicited by digital pressure to points of tenderness in muscle nearby the scapula (Edeiken, 1936).

The physician who gave the greatest contribution to the knowledge of what is now called MPS was John Kellgren. He was Professor of Rheumatology at Manchester University but during the 1940s, he also conducted many research studies on muscle pain pathophysiology. His publication described the referred pain distributions evoked by injecting 0.1 to .03 c.c. of 6% saline into muscles and spinal ligaments (figure 1.8) (Kellgren, 1938).

Kellgren moved his attention from the local pain, induced by the injection, to what he called the zone of pain referral and adopted this experimental observation directly to enhance the clinical evaluation of patients with muscle pain. He stated "*The distribution of pain from normal muscles guided me to the muscles from which spontaneous pain may have arisen. Such muscles always presented tender spots on palpation and pressure on these spots reproduced the patient's pain*."(Kellgren, 1938).

He concluded in accordance with previous authors, that pain was a consequence of localized nerve hyperactivity in specific regions of the affected muscles. Additionally, he proved that pain can be reduced by injecting an anaesthetic solution at the tender sites.

In line with Kellgren' findings, Kelly (1941) during a cases-series on 200 subjects with fibrositis, noticed both the palpable hardness of the "nodule" associated with the muscle tenderness and the distant referral of pain in the involved muscles. Kelly developed the concept that fibrositis was a functional neurologic disorder originating at the myalgic lesion (Kelly, 1941). Likewise, in

the same period, Good (1942) examined 500 cases of myalgia and described both the myalgic spots and the referred pain for 20 muscles (Good, 1942).
It is at that time that Janet Travell, an American physician, undertook a systematic investigation of this disorder (Travell et al., 1942). She expanded the research' line initiated by Kellgren and introduced, as proposed by an American orthopaedic surgeon, the term trigger point. A case series of 58 subjects with musculoskeletal pain in the upper quadrant was used to widely describe the clinical findings related to MTrPs such as precipitating factors, number of MTrPs for each patient, and distribution of the referred pain. Interestingly, she noticed that apart from infraspinatus' and serratus posterior' MTrP the pain patterns did not clearly follow the somatic reference zones of the spinal segments (Travell et al., 1942). Ten years later together with Seymour Rinzler, she also determined the pain patterns of MTrPs in 32 skeletal muscles and compiled anatomical charts of MTrPs (Travell and Rinzler, 1952).

The medical terminology was subsequently improved by adding the term "myofascial" to point out that pain arises both from muscular and connective tissues, thus the related medical diagnosis was named MPS.
Between 1942 and 1992, Travell published more than 15 scientific papers and four books. Together with her colleague David Simons, she described the clinical presentation of trigger points in many different muscles, and provided diagrams of both their locations and the referred pain' patterns. All this information together with many treatment techniques, such as stretch and spray or injection, were included in the well-known publication: *The Trigger point Manual (Travell and Simons, 1983)*. These books are still considered the most extensive and authoritative publications on MPS and introduced this diagnosis to the medical community (Tough et al., 2007).

Figure 1.8: Diagram of the distribution of referred pain after a saline injection of the multifidus muscle at L5 level (Adapted from Kellgren, 1938).

More recently, other authors have contributed to further develop the understanding of MTrPs. During the 80s, Karel Lewit explored the manual treatment of MTrPs, as he was particularly interested in the interaction between joint dysfunctions and trigger points (Dostal et al., 1978, Janda et al., 1979, Lewit and Simons, 1984). Chang-Zern Hong proposed the first animal model, contributed to several publications on animal pathophysiology and was also a pioneer of the dry needling technique (Hong, 1994b, Hong, 1996,

Hong, 2002, Hong and Hsueh, 1996, Hong and Simons, 1998, Hong, 1994a). After 2000, Hong-You Ge led several experimental investigations and provided important contributions to address the enigmatic MTrP' pathophysiology (Ge et al., 2012, Ge et al., 2014, Ge et al., 2008a, Ge et al., 2008b). Jan Dommerholt, who wrote several critical syntheses of the recent scientific literature provided an outstanding contribution for researchers in the field of myofascial pain (Bron and Dommerholt, 2012, Dommerholt, 2005, Dommerholt, 2008, Dommerholt, 2010, Dommerholt, 2011b, Dommerholt, 2011a, Dommerholt et al., 2006, Dommerholt et al., 2016, Dommerholt and Gerwin, 2015, Dommerholt et al., 2015a, Dommerholt and Huijbregts, 2011, Dommerholt et al., 2015b, Fernandez-de-las-Penas and Dommerholt, 2014, Gerwin et al., 2004). Finally, Cesar Fernandez-de-las-Penas promoted several clinical trials to investigate the effectiveness of manual therapy and dry needling for MPS (Alonso-Blanco et al., 2012, Arias-Buria et al., 2015, Bodes-Pardo et al., 2013, Cantarero-Villanueva et al., 2012, Fernandez-Carnero et al., 2010, Fernandez-Perez et al., 2012, Iglesias-Gonzalez et al., 2013, Llamas-Ramos et al., 2014, Mejuto-Vazquez et al., 2014, Renan-Ordine et al., 2011). The international literature is still growing and contributing to the body of knowledge. However, despite the large volume of research, the methodological quality of the studies is frequently poor and the need for a validated pathway to diagnose MTrP is underestimated.

1.4 EPIDEMIOLOGY

The literature suggests that MTrPs are extremely common and can be considered both a primary cause of a MPS or a secondary pain generator in patients affected by a principal musculoskeletal disorder (Fricton, 1991, Giamberardino et al., 2011, Zuil-Escobar et al., 2016, Lluch et al., 2015, Roach et al., 2013). Unfortunately, accurate information on the prevalence of MTrPs and MPS is lacking. To properly address this epidemiological issue, it is important to investigate the MPS prevalence in general populations as well as the MTrPs' prevalence in selected conditions. In the latter case, it may be important also to distinguish active and latent MTrPs. Considering the

variability of the criteria used to diagnose MPS and low reliability for the MTrPs' diagnostic criteria, this should be considered a very complex and challenging goal (Lucas et al., 2009, Myburgh et al., 2008, Tough et al., 2007). A few cross-sectional studies on musculoskeletal pain that selected healthy subjects according to their occupational/professional category, can be identified in the scientific literature but some of them do not conform properly to the MTrP's definition (Schiffman et al., 1990, Fröhlich and Fröhlich, 1995, Sola et al., 1955, Chaiamnuay et al., 1998, Fernandez-de-las-Penas et al., 2012, Celik and Kaya Mutlu, 2012).

A first attempt to investigate the presence of muscle tenderness was conducted in a study of 269 randomly selected female student nurses with or without pain symptoms (Schiffman et al., 1990). Muscle tenderness was identified in the craniocervical region, using a palpation protocol to identify tender points. In neck muscles, tender points were recognized in 35% of the right splenius capitis muscles and in 33% of the right upper trapezius muscles. In masticatory muscles, tender points were recognized in 54% of the right masseter muscles, in 43% of right anterior temporalis muscles, and in 40% of right medial pterygoid muscles (Schiffman et al., 1990). Similarly, a population of 200 asymptomatic adults from Air Force Personnel were screened for a focal tenderness in the shoulder muscles. The focal tenderness (i.e. hypersensitive areas during manual palpation) was fully comparable to a latent MTrP and the authors reported a rate of 54% for females and 45% for males (Sola et al., 1955). Additionally, in 25% of the subjects, it was possible to elicit referred pain by palpation. Results of both studies should be considered with caution as MTrP's diagnostic criteria was not reported.

Two German physicians investigated the presence of latent MTrPs in the lumbo-gluteal region among 100 healthy subjects. They observed latent MTrPs in the following muscles: quadratus lumborum in 45% of the subjects, gluteus medius in 41%, iliopsoas in 24%, gluteus minimus in 11%, and piriformis in 5% (Fröhlich and Fröhlich, 1995). Again, a study about the musculoskeletal disorders in villagers from rural Thailand, has indicated MPS

in 6.3 % of 431 subjects reporting pain during the previous week (Chaiamnuay et al., 1998).

In order to explore the relationship between MTrPs and work related activities, a recent study investigated the MTrPs prevalence in blue-collar and white-collar workers. The authors hypothesized that different work activities or physical load can determine the MTrPs' activation. Although the sample was very small (n = 35), it was interesting to note that the two groups exhibited a similar number and distribution of active and latent MTrPs. The observed prevalence was high, with 6 ± 3 blue-collar and 6 ± 4 white-collar workers showing active MTrPs (table 1.1 and 1.2) (Fernandez-de-las-Penas et al., 2012).

Again in 2012, an original study conducted at the Istanbul University (School of Physical Therapy and Rehabilitation) observed for the first time, a close relationship between latent MTrPs in periscapular muscles and depression in healthy people (Celik and Kaya Mutlu, 2012). Given that latent MTrPs can develop into active MTrPs, it seems relevant to carefully assess muscles for MTrPs presence not only in patients with common musculoskeletal disorders. Finally, due to the paucity of data on the MTrP prevalence for lower limbs, a study on 220 healthy volunteers was conducted (Grieve et al., 2013a). The principal aim was to establish the prevalence of latent MTrPs in triceps surae. Interestingly, to compare findings, the MTrP prevalence in upper trapezius muscles was also examined. Prevalence of latent MTrP in triceps surae was recorded in six different locations and its rate ranged from 13 % to 30%; the highest prevalence was recorded for the medial portion of the left gastrocnemius muscle. The MTrP prevalence of the left upper trapezius muscle was 23% while for the right, it was 20%. Latent MTrPs in the soleus muscle have been associated with a limitation of the dorsiflexion of the ankle and calf cramps (Grieve et al., 2011) (Kim et al., 2005, Ge et al., 2008b). Similarly, latent and active MTrP have been associated with several musculoskeletal conditions (Bron et al., 2011b, Calvo-Lobo et al., 2016, Roach et al., 2013, Fernandez-de-las-Penas et al., 2006a) or neurophysiological impairments (Ge et al., 2012, Ge et al., 2014). These results contribute to increased knowledge of the prevalence of MTrP in a

healthy population and suggest that clinicians should carefully examine the role of MTrPs in a clinical population as well as healthy subjects. Moreover, a few reports (Bron et al., 2011b, Fernandez-de-las-Penas et al., 2007, Schiffman et al., 1990, Roach et al., 2013) have been published on the prevalence of MTrPs among selected patient groups and once again, a high prevalence is reported, suggesting that the MTrP's nature also as an associated phenomenon. Results from these studies are summarized in table 1.3.

In an internal medicine group practice, 54 out 174 patients complaining of pain were examined and in 30% of the cases, their painful symptoms were related to the presence of a MTrP (Skootsky et al., 1989). Notably, patients with upper body pain were more likely to have a MPS diagnosis.

Again, among 164 patients with chronic head and neck pain, 55% had a primary diagnosis of MPS, and in 97 patients examined at an Orthopaedic clinic, 70% presented with an active or latent MTrP (Fricton et al., 1985, Fröhlich and Fröhlich, 1995).

Fishbain and his collaborators (1986) reported a similar prevalence in 283 patients presenting to a Comprehensive Pain Centre of the University of Miami School of Medicine. MPS was the primary condition in 85% of the patients (Fishbain et al., 1986), the diagnosis was established by two physicians that applied the diagnostic criteria proposed by Simons and Travell (Travell and Simons, 1983). Gerwin examined 96 patients at a Pain Medicine Centre. Surprisingly, he reported that MTrPs, in 93% of the subjects, were at least partially responsible for the pain symptomatology and were the primary cause of pain in 74% of the subjects (Gerwin, 1997)

Table 1.1: Number of blue-collar workers (n = 16) with active or latent MTrPs for 15 muscles on both sides (Adapted from Fernandez-de-las-Penas et al., 2012)

	Right side	Left side	Right side	Left side	Right side	Left side
	Temporalis muscle		**Masseter Muscle**		**Upper trapezius muscle**	
Active MTrPs (n)	1	1	0	0	11	9
Latent MTrPs (n)	4	2	10	10	2	4
No MTrPs (n)	11	13	6	6	3	3
	Sternocleidomastoid muscle		**Splenius capitis muscle**		**Oblique capitis inferior muscle**	
Active MTrPs (n)	1	3	6	5	4	2
Latent MTrPs (n)	7	9	3	3	5	6
No MTrPs (n)	8	4	7	8	7	8
	Levator scapulae muscle		**Scalene muscle**		**Pectoralis major muscle**	
Active MTrPs (n)	2	4	1	2	3	3
Latent MTrPs (n)	3	3	4	4	7	6
No MTrPs (n)	11	9	11	10	6	7
	Deltoid muscle		**Infraspinatus**		**Ext. carpi radialis brevis muscle**	
Active MTrPs (n)	2	3	7	6	4	2
Latent MTrPs (n)	3	4	8	9	5	7
No MTrPs (n)	11	9	1	1	7	7
	Ext. Car. radialis longus muscle		**Ext. digitorum communis muscle**		**Supinator muscle**	
Active MTrPs (n)	4	2	1	0	2	1
Latent MTrPs (n)	5	4	6	7	8	9
No MTrPs (n)	7	9	9	9	6	6

Table 1.2: Number of white-collar workers (n = 19) with active or latent MTrPs for 15 muscles on both sides. (Adapted from Fernandez-de-las-Penas et al., 2012)

	Right side	Left side	Right side	Left side	Right side	Left side
	Temporalis muscle		**Masseter Muscle**		**Upper trapezius muscle**	
Active MTrPs (n)	1	1	1	3	12	12
Latent MTrPs (n)	4	9	10	11	7	6
No MTrPs (n)	14	9	8	5	0	1
	Sternocleidomastoid muscle		**Splenius capitis muscle**		**Oblique capitis inferior muscle**	
Active MTrPs (n)	4	4	4	3	6	6
Latent MTrPs (n)	10	10	2	3	6	5
No MTrPs (n)	5	5	13	13	7	8
	Levator scapulae muscle		**Scalene muscle**		**Pectoralis major muscle**	
Active MTrPs (n)	7	6	3	4	1	1
Latent MTrPs (n)	2	5	6	6	12	15
No MTrPs (n)	10	8	10	9	6	3
	Deltoid muscle		**Infraspinatus**		**Ext. carpi radialis brevis muscle**	
Active MTrPs (n)	2	1	4	6	4	3
Latent MTrPs (n)	4	5	10	10	7	9
No MTrPs (n)	13	13	5	3	8	7
	Ext. Car. radialis longus muscle		**Ext. digitorum communis muscle**		**Supinator muscle**	
Active MTrPs (n)	5	3	4	1	6	3
Latent MTrPs (n)	7	7	7	9	6	8
No MTrPs (n)	7	9	8	9	7	8

Table 1.3: Prevalence of MTrPs among selected patient groups.

Authors	Practice	Diagnosis	Number studied	% with MTrP
Skootsky et al.,1889	Medical	n/a	172	30
Fricton et al., 1985	Head and Neck Pain Clinic	n/a	164	55
Fishbain et al., 1986	Comprehensive Pain Center	n/a	283	85
Frohlich and Frohlich, 1995	Orthopaedic Clinic	n/a	97	93
Gerwin, 1997	Pain Medicine Center	n/a	96	70
Fernandez-de-la-Penas et al., 2007	N/A	Neck pain	20	100
Bron et al., 2011	Physiotherapy	Shoulder pain	72	100
Roach et al., 2013	Physiotherapy	Patellofemoral pain	52	97

Subsequently, in a blind controlled study including 20 neck pain patients and 20 healthy subjects, at least three MTrPs were identified in patients. On the other hand, all the control healthy subjects exhibited latent MTrPs (Fernandez-de-las-Penas et al., 2007). Similarly, an observational study conducted at a primary care practice for physical therapy, revealed MTrPs in all patients with shoulder pain. The mean number of active MTrPs per subject was 6, and infraspinatus was the muscle most frequently involved (77%) (Bron et al., 2011b). The authors also pointed out a moderate correlation between the number of MTrPs and disability measured with a multidimensional scale (Disabilities of the Arm, Shoulder, and Hand scale). Also, in a selected group of patients with the patellofemoral pain, a high prevalence for MTrP was observed (Roach et al., 2013). Bilateral MTrPs were identified in gluteus medius and quadratus lumborum muscles and a related reduction of the hip abduction' strength was also observed.

Large-scale epidemiological studies in the general population for MPS are not available. Current data are from small, selected groups and the biggest sample investigated was from the study of Fishbain, who examined 283 consecutive admissions to a pain centre. Additionally, in the aforementioned epidemiological studies, the diagnostic criteria for the MPS diagnosis were not clearly reported and an adequate training for the manual palpation of a MTrP was not provided to the assessors.

The selected studies showed wide-ranging prevalences for active and latent MTrPs (30% to 100%). The observed variability for the prevalence can be partially explained by diversity of the selected groups. In most of the studies, the main complaint was pain and for subjects with a confirmed MPS diagnosis, no information on comorbidity was considered. Thus, it is not possible to establish if MPS was the principal diagnosis or an associated disorder. This is an important issue when establishing the clinical relevance of a diagnosis and subsequently, for the planning of a proper treatment. Only a few researchers (Bron et al., 2011b, Fernandez-de-las-Penas et al., 2007,

Roach et al., 2013) selected groups of patients using a medical diagnosis as inclusion criteria. They enrolled subjects with non-traumatic shoulder pain (Bron et al., 2011b), mechanical neck pain (Fernandez-de-las-Penas et al., 2007) , and patellofemoral pain (Roach et al., 2013) using detailed exclusion criteria. Surprisingly in both of the studies, the prevalence approached 100% and each subject exhibited several MTrPs.

This suggests that muscles are an important source of pain in the three conditions investigated and it is possible to speculate that active MTrPs are a very common cause of pain and dysfunction in many non-specific musculoskeletal disorders, like for example, low back pain. On the other hand, a poor agreement on the diagnostic criteria and differences in training of examiners has been reported. Also, all the investigators during the experimental procedures were focused on only one medical diagnosis, which was indeed the MPS. These elements could lead to a significant number of false positives and thus, an overestimation of the MPS' prevalence. There is a need, as already remarked upon by other authors (Simons, 2004, Shah et al., 2015), to improve reliability and validity of the MTrP' diagnostic criteria.

1.5 AETIOLOGY AND PATHOPHYSIOLOGY

Before the mid-1990s, key elements regarding the pathophysiology of MTrPs were unrecognized. Subsequently, research studies have made the pathophysiology of MPS much better understood. Three main hypotheses have been provided: energy crisis theory, muscle spindle concept and the motor endplate hypothesis (Simons, 1996). Each of them provides elements that can explain part of the symptoms and signs of MPS. Later Simons (1999) presented an integrated hypothesis that Gerwin (2004) subsequently developed further by the inclusion of new experimental data accounting for additional aspects of muscle pathophysiology. The integrated hypthesis combines several important electrophysiological and histopathological research findings.

1.5.1 Energy Crisis Theory

Simons and Travell introduced the energy crisis theory for the first time in a paper published in the journal Pain in 1981 (Simons and Travell, 1981) and then it was updated with the inclusion of some experimental evidence, in 1993 (Lindblom et al., 1993). It was developed with regard to a few aspects considered characteristic of the MTrP and with the the intention of providing a robust rationale for the MPS' pathophysiology. The following elements were considered as starting points for the authors' speculation.

1. Subjects affected by MTrPs at rest do not show any motor units' action potentials travelling along the taut band.
2. It is well-known among clinicians that MTrPs are usually induced by muscle overload, especially low level sustained muscle activity
3. the evident focal hyperalgesia of the MTrP
4. the efficacy of treatments aimed to stretch or compress the MTrP taut band

The proposed theory enclosed a vicious cycle (figure 1.9) that starts with a lesion of the sarcoplasmic reticulum or the sarcolemma and the release of calcium in the cytoplasm. The exact mechanism responsible for the described lesion was not reported and only mentioned as a generic trauma.

The non-physiological delivery of calcium would activate the actin and myosin contractile activity in a limited number of fibres (i.e taut band). This sustained shortening of the fibres will induce at the same time an increase of the local metabolism and a compression of capillaries that supply oxygen and nutritional substances. The simultaneous increased metabolism and the impaired metabolic intake could induce the so-called energy crisis, during which a release of sensitising substances is postulated. Finally in this dysfunctional context the calcium pump, that returns calcium into the sarcoplasmic reticulum, cannot work properly due to the lack of adenosine triphosphate (ATP). This completes the vicious cycle, where an abnormal

quantity of calcium remains in the muscle fibres cytoplasm promoting a further shortening of the fibres. The proposed hypothesis fits with the absence of motor unit action potentials as the described contracture should be considered endogenous and not nerve-initiated, the mechanical sensibility of the synaptic cleft region that release calcium due to a trauma, the release of sensitizing substances that explain the local hyperalgesia, and finally the efficacy of the stretching techniques in reducing pain.

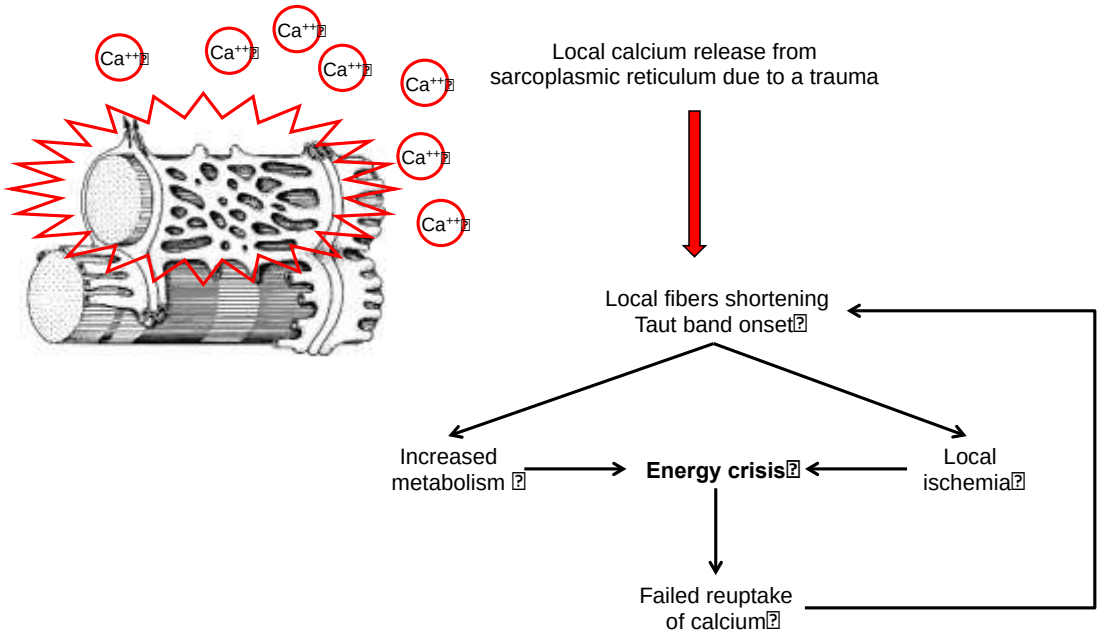

Figure 1.9: Energy crisis hypothesis and the vicious cycle of events that contribute to MTrP' manifestation.

1.5.2 - Muscle spindle concept and the motor endplate hypothesis.

Electromyography investigations of different painful muscle syndromes have produced conflicting results and the concept of a pain-spasm-pain cycle is not fully supported by physiologic and clinical evidence. Two editorials in important scientific journals (Olesen and Jensen, 1991, Johnson, 1989) reviewed this issue and remarked that it was not clear that increased EMG

activity did not account for the muscle tenderness. Similarly, in 1957, Travell described a high-frequency firing (Travell, 1957) from MTrPs, but later the same author, together with Simons, published in *The Trigger point Manual* (Travell and Simons, 1983) that MTrPs did not show EMG resting activity.

Hubbard and Berkoff (1993) attempted to clarify this issue by recording needle EMG signals simultaneously from the MTrP site and from an adjacent non-painful site of the same muscle (Hubbard and Berkoff, 1993). They included in their study, three groups of subjects with active or latent MTrPs: normal subjects, tension headache patients and fibromyalgia patients. Spontaneous EMG activity (SEA) was evident from the MTrPs of all normal subjects and patients, while no SEA was recorded from the control sites. In all subjects, the occurrence of the SEA corresponded to the manifestation of a painful symptomatology. MTrPs' mean EMG amplitudes for normal subjects were significantly lower than for patients, but the EMGs were always higher at MTrP' sites than in the control site, especially for the two patients' groups. The authors concluded that the source of the EMG activity in the vicinity of the MTrPs were the dysfunctional muscle spindles (Hubbard, 1996, Hubbard and Berkoff, 1993). To support their hypothesis, the authors commented that the EMG activity was not localised enough to be generated in the endplate region. Even the waveform morphology was not considered typical for an endplate activity. Additionally, and by the fact that the electrical phenomenon showed a localized nature, it was possible to rule out a muscle spasm or a metabolic disorder. It was also speculated that the aetiology was related to prolonged spindle tension that becomes painful by distending or chemically sensitizing the spindle' capsule. The sympathetic innervation of the intrafusal fibres was put forward as an explanation for the autonomic symptoms associated with MTrPs (Hubbard and Berkoff, 1993).

These assertions opened a debate among researchers. Simons interpreted the experimental findings differently and came to opposite conclusions. The localizations of EMG activity reported in the study of Hubbard and Berkoff,

according to Simons, was thought to be similar to that previously observed in a scientific paper on the source of motor endplate potentials (Wiederholt, 1970). Additionally, he also remarked that even the observed waveforms corresponded to atypical EMG records from the endplate region, defined as “Noise” (Buchthal and Rosenfalck, 1966). This convinced Simons to study in depth, the EMG signals from MTrPs and particularly, the waveforms of the SEA. Initially, Hubbard and Berkoff (1993) reported that a muscle affected by a MTrP has several sites (named active loci) where it was possible using the needle EMG, to record low voltage, continuous noise-like potentials and an intermittent spikes (i.e. SEA) for as long as the needle was not removed (Hubbard and Berkoff, 1993) . They focused their attention on mean EMG amplitude and spikes without going into more details of the signals. Surface EMG signals from a MTrP were recorded at slow speed and the general pattern of activity was appreciated but details were not available (figure 1.10A).

Many large spikes were clearly visible but the small-amplitude SEA cannot be distinguished clearly and the waveform of SEA remains hidden in the electromyographic path. Then Simons decided to record sEMG signals from MTrPs at higher speed and succeeded to show a marked difference between the SEA and the higher amplitude spikes (figure 1.10B) (Simons et al., 1999). The SEA looked like a low amplitude noise while the high amplitude spikes were sharp, initially-negative and diphasic (Simons et al., 1999).

Simons commented that according to his experience, the SEA of MTrPs is located in the endplate region and not in the taut band outside of the endplate region (Simons et al., 1995c, Simons et al., 1995a). Again, he speculated that in accordance to a few old physiological studies, the SEA arises froma dysfunctional endplate. Indeed, Liley in 1956, observed that a mechanical stress (i.e. vibration at the endplates site, stretching the motor nerve, dimpling the muscle surface) applied to the endplate region, increases the frequency of the post-junctional membrane potentials and could start an abnormal EMG pattern, comparable to the SEA (Liley, 1956). Later, a

preliminary study on the effectiveness of botulinum A toxin to reduce pain in patients with MTrPs, further supported the endplate hypothesis (Yue, 1995).

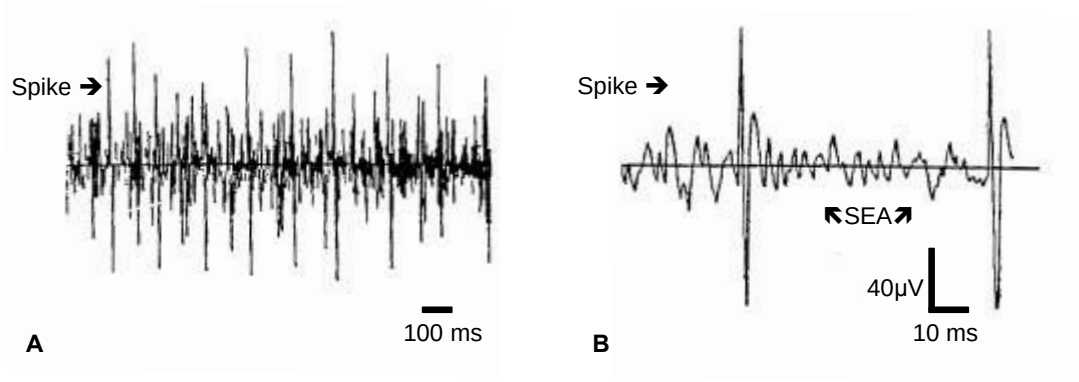

Figure 1.10: EMG signals at an MTrP' site in the upper trapezius muscle. A) EMG signals recorded at slow speed, B) EMG signals recorded at high speed (Adapted from Hubbard & Berkoff, 1993).

The nature of this localized atypical EMG activity remained partially unsolved. Although it seems improbable that muscle spindles were the site of the described electrical activity, it was not possible to exclude an important contribution of muscle spindles to the MTrPs' phenomena. The issue of whether the SEA, finally recognized by electromyographers as a type of endplate noise, arose from normal or from dysfunctional endplates became critical to validate this hypothesis. As well as its correlation with the MTrP should be confirmed.

A first attempt to solve this issue was in 1995 (Simons et al., 1995b), during a preliminary study, but final results were published subsequently, in March 2002 (Simons et al., 2002). Investigators examined the SEA in different sites of muscle affected by MTrP, using needle EMG. Three different sites were considered for the SEA detection: in the MTrP, in the endplate zone outside of the MTrP, and in the taut band outside the endplate zone (figure 1.11).

All sites were examined systematically by inserting an EMG needle in three divergent tracks (30, 45, 60 degrees to the skin surface). Needle

advancement was slow and gentle, stopping whenever the SEA with or without spikes was observed and whenever the EMG needle had advanced 1.5 mm. Eleven muscles in 10 subjects, showed SEA to be four times more frequent in the MTrP' site than in the endplate zone outside of the MTrP' site (table 1.4). Additionally, no SEA was observed outside the endplate' zone further supporting the hypothesis that the SEA arises from the endplate' zone.

The validity of this finding was also supported by a similar animal' study conducted on the biceps femoris muscle of rabbits (Simons et al., 1995d). A review of physiology' literature also indicated that the waveform of the SEA was considerably different from normal endplate potentials and that the abnormal waveform pattern of the SEA was probably related to an increased release of ACh from the postjunctional membrane (Simons, 2001).

Two possible mechanisms were considered as explanations of the SEA phenomenon and the abnormal release of ACh. The first assumes that the SEA's sEMG' pattern may appear due to the release of ACh packets because of the mechanical stress caused by the EMG needle. In this case, before the needle insertion, the endplate would have been producing normal endplate potentials. Thus, if this was the case, it was possible to speculate that some of the endplates associated with a MTrP, were more sensitive to mechanical stress by the needle than an endplate that was not associated with the MTrP (Simons et al., 2002). This rationale is well supported by previous experimental findings focusing on the effect of mechanical, chemical and immunological stimuli on the endplate physiology (DeBassio et al., 1971, Heuser and Miledi, 1971, Ito et al., 1974).

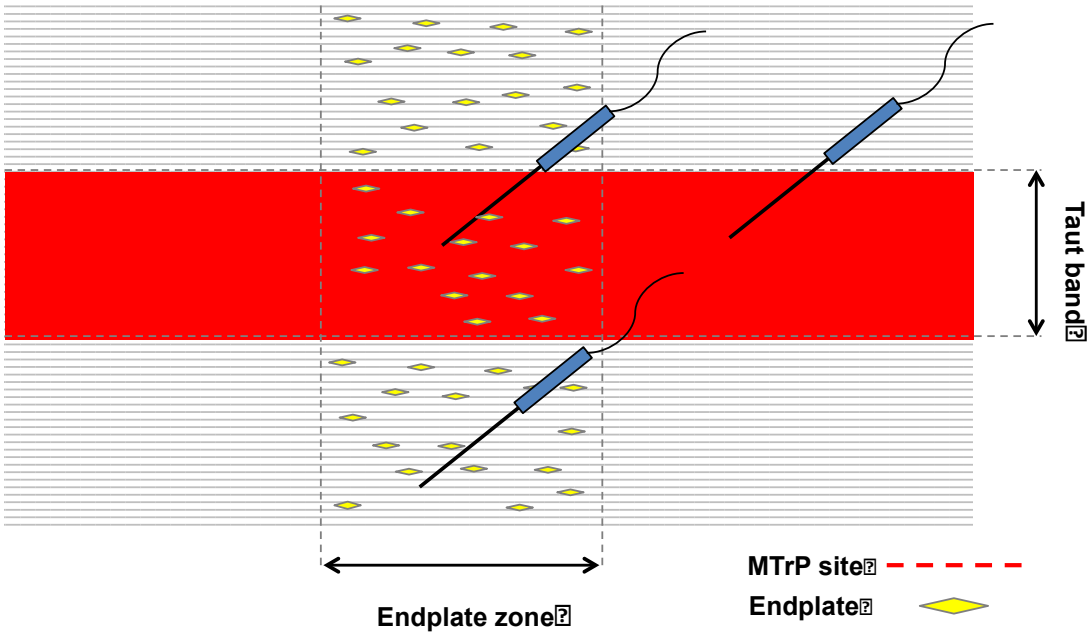

Figure 1.11: The illustration indicates the sites selected to investigate the SEA presence in a muscle affected by a MTrP. The muscles were systematically explored inserting the needle in the MTrP, in the endplate zone 1-2 cm away from the MTrP, and in the taut band (Adapted from Simons, Hong, & Simons, 2002).

The second possibility is obviously that the observed SEA could have been present as a result of a pathophysiologic condition that is related to the clinical manifestation of MTrP. Further research is needed to confirm this latter hypothesis as the results from the Simons et al study neither refuse nor prove this possibility. The SEA, currently named by experts in this field as endplate noise (EPN), should be demonstrated the absence of needle stimulation. As reported in table 4 the tenderness of the MTrP was commonly associated with the SEA with or without spikes but only exceptionally when only spikes were recorded. Moreover, the SEA was never detected in taut band sites.

Table 1.4: SEA recordings during 264 needle advances in 11 muscles. Number of observations of the SEA only, the SEA with spikes, and only spikes. Note that the SEA was not detected in the taut band sites considered. SEA, spontaneous electrical activities, (Adapted from Simons, Hong, & Simons, 2002).

EMG activity detected	MTrP sites	Endplate site	Taut band sites
SEA without spikes	21	7	0
Present*	10	4	0
Absent°	1	7	11
SEA with spikes	14	2	0
Present*	7	2	0
Absent°	4	9	11
Spikes only	1	12	2

* Number of muscles in which SEA only or SEA with spike was detected. °Number of muscles in which SEA only or SEA with spike was not detected.

A few methodological limitations should be taken in to account when asserting a spatial relationship between the SEA and the MTrP. The alteration of pain perception in subjects due to the experimental procedures and the absence of standardized procedures to estimate pain during the needle advancement, are among these. Nevertheless, modifications of the motor endplate potentials in association with pain, have been already reported by previous investigators (Brown and Varkey, 1981, Wiederholt, 1970, Jones et al., 1955).

1.5.3 The integrated trigger point hypothesis and his expansion.

The integrated trigger point hypothesis classifies the MTrP as a neuromuscular disease and postulates that the endplate dysfunction, and the excessive release of Ach, are central events (figure 1.13) (Simons, 2004). It was originally proposed in 1999 by Simons in the second edition of his textbook “Myofascial Pain and Dysfunction: The Trigger Point Manual” (Simons et al., 1999). It is essentially an original update of the “Energy Crisis Hypothesis” and combines electrodiagnostic and histopathological evidence (Simons and Travell, 1981). This hypothesis has been further developed according to some recent findings and it is now the most complete rationale for the MTrP pathophysiology (Gerwin et al., 2004). The hypothesis, named in a few papers as the expanded trigger point hypothesis, is a positive-feedback cycle that includes a few activating events, and where the starting point is an acute or persistent muscle overload (figure 1.12).

According to the authors, it can be an unaccustomed or excessive eccentric/concentric muscle activity that leads initially to ischemia and afterwards to the endplate dysfunction (Treaster et al., 2006, Huang et al., 2013, Bron and Dommerholt, 2012). To explain the localized manifestation of MTrP, indeed it involves only a limited amount of contracted fibres within a muscle (i.e. taut band), the Cinderella Hypothesis has been suggested (Hagg, 1991). The Cinderella Hypothesis states that muscle pain can be

caused by selective overloading of type I muscle fibres, driven into a degenerative process as a consequence of long-standing activation, with a short recovery time. According to Hägg, who developed the Cinderella Hypothesis, low-level sustained contraction can engage a fraction of the motor units that show an inefficient process of motor units' substitution and which is important to protect the muscle from excessive fatigue (Hagg, 1991). The overuse of a limited group fibres will lead initially, to energy depletion and subsequently, to tissue injuries with the release of sensitizing substances (Gerwin et al., 2004).

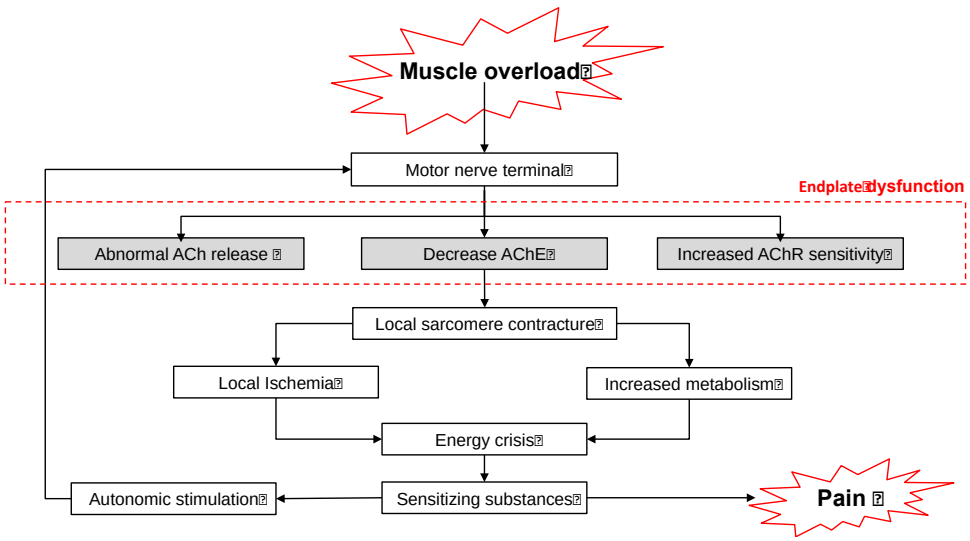

Figure 1.12: A flow chart of the integrated trigger point hypothesis (Adapted from Gerwin et al., 2004).

Additionally, according to some recent studies, a persistent mechanical stress (i.e. hypertonicity or stretching) may augment the ACh release (Grinnell et al., 2003, Chen and Grinnell, 1997). Chen and Grinnell showed that a 1% increase in muscle stretch at the motor endplate induced a 10% increase in ACh release. This event has been related to a tension on the integrins (i.e. cell adhesion molecules) in the presynaptic membrane at the motor endplates, that can theoretically trigger an ACh release, which does not require Calcium (Kashani et al., 2001, Chen and Grinnell, 1997, Grinnell et al., 2003). The integrins are a family of cell adhesion receptors that mediate the attachment between a cell and its surroundings, such as other cells or the extracellular matrix. In this context, the sodium channels of the sarcoplasmic reticulum are conditioned by the presence of ACh, and increase the intracellular calcium levels. This will further induce a local muscle contracture (i.e. taut band) in the absences of motor unit potentials.

The proposed aetiology and the related mechanism is more convincing than the “Energy Crisis Theory”. Indeed, it was initially proposed in relation to a generic trauma, with no clear link to clinical practice, which suggests that MTrPs are induced by a long-lasting low intensity contraction or high intensity repeated contractions. As a consequence of this long-lasting local contracture, it is reasonable to think also that some myosin filaments of the taut band can be locked in the Z-band of the sarcomere. The regular physiology of muscle contraction that includes myosin sliding, become then altered, and some sarcomeres can not restore their resting length. Once again, the shortened sarcomeres will further contribute to the local circulation deficit (Dommerholt and Huijbregts, 2011, Dommerholt et al., 2006). The subsequent hypoxia will induce the release of sensitizing substances and activates the muscle nociceptors. The result will be a decrease of energy supply and a simultaneous increase of metabolic demand. This chronic energy deficit would also explain the described finding of ragged red fibres and swollen mitochondria, which are typically observed in the nerve terminal of patients with muscle pain (Larsson et al., 2000).

A few histological studies provide an additional support for the pathophysiology described above (Simons and Stolov, 1976, Windisch et al., 1999, Mense et al., 2003, Reitinger et al., 1996). A biopsy performed by Simons and Stolov (1976) in canine gracilis muscle, highlighted for the first time multiple contraction knots in several fibres, and it showed many shortened sarcomeres in the proximity of the MTrP, as well as many lengthened sarcomeres distally to the MTrP (Simons and Stolov, 1976). Also, Windisch and colleagues in post-mortem biopsies, described similar histological alterations (Windisch et al., 1999). In support of this pathophysiological process, a recent study also demonstrated that hypoxia can determine a spontaneous quantal and non-quantal release of ACh at mouse' motor endplates (Bukharaeva et al., 2005). The mechanical and chemical stressful events can induce a sensitization of the peripheral nerve endings, autonomic nerves, and second order neurons. This will lead to a central sensitization and to the formation of new receptive fields that will explain the clinical manifestation of referred pain and hyperalgesia.

The recent findings of Jay Shah (2008), a physiatrist at the Clinical Center of the National Institutes of Health (NIH) in Bethesda, have been considered a milestone in the understanding of the MTrP' sensory component and also represented an important contribution for the development of the integrated trigger point hypothesis (Simons, 2008). His NIH project applied a notable technique of placing two tubes inside a 30-gauge acupuncture needle, with a microdialysis membrane between the ends of the open tubes and a 10 μm opening at the tip of the acupuncture needle (Shah et al., 2005, Shah et al., 2008). The aim was to sample the unique biochemical milieu of substances related to pain and inflammation in muscle tissue, with and without MTrPs. The special needle was inserted into three different sites of the trapezius muscle: active MTrP of patients, latent MTrP in pain-free subjects, and normal muscle (MTrP-free) in healthy subjects. Samples were obtained before the needle movement, during the needle advancement involving local

twitch responses, and after the local twitch response. Samples of dialysate were analysed for electrolytes, pH, muscle metabolites, inflammatory mediators, arachidonic acid derivatives, cytokines, and neurotransmitters. The concentrations of neuropeptides substance P, calcitonin gene-related peptide, bradykinin, 5-hydroxytryptamin/serotonin (neurotransmitters), TNF-α and IL-1 (cytokines) were significantly higher in active MTrPs than in latent MTrPs and normal muscle (healthy subjects). Also, the pH levels were lower in the active MTrPs than in latent MTrPs and normal muscles. In addition, there were no overall differences between subjects with latent MTrPs and normal subjects (MTrP-free). Finally, after the local twitch response at the active MTrP, the europeptides substance P and the calcitonin gene-related peptide concentrations decreased significantly. These results showed for the first time, significant biochemical differences between subjects with and without MTrPs, for a number of substances. A few interactions between the observed substances help to explain the local hyperalgesia, the endplate dysfunction, as well as the referred pain of MTrPs. First, the bradykinin is able to activate and sensitize muscle nociceptors, leading to a inflammatory hyperalgesia (Verri et al., 2006). Moreover, the bradykinin stimulates the release of TNF-α that activates the production of cytokines, especially the IL-8 that can induce once again, hyperalgesia. The observed acid pH, probably a consequence of the hypoxia and the injured fibres, inhibit AChE' activity and increased the calcitonin gene-related peptide level, which can enhance the release of ACh from motor endplate and at the same time decrease the effectiveness of AChE (Fernandez and Hodges-Savola, 1996, Hodges-Savola and Fernandez, 1995). Again, calcitonin gene-related peptide up-regulates the ACh receptors, providing more binding opportunities for ACh. The state of the ACh receptors and the ACh concentration are considered critical to explain the EPN described by Simons and his collaborators (Simons et al., 1995d, Simons et al., 1995c, Simons, 2001). The Shah' findings (2008) have provided an important contribution to the MTrP' physiopathology but a few methodological limitations should be noted. The sample size was small (n = 9) and composed of healthy participants from a

working population. Also, the reported alterations of the biochemical milieu are consistent with inflammation due either to muscular tissue damage or to dysfunctional peripheral nerve function (Mense, 2009, Chiu et al., 2012).

More recently, Mense completed an interesting study by developing an experimental animal model to induce MTrP in rats (Mense et al., 2003). The study was undertaken to test if a local increase of ACh leads to an abnormal contraction that causes the formation of contraction knots. A small quantity of acetylcholinesterase inhibitor was injected into a limited area of gastrocnemius muscle of 22 rats. Subsequently, to simulate an intense muscle activity, the nerves of the muscle were electrically stimulated for 30-60 minutes. The data supported the initial hypothesis and showed that an increase of ACh in the endplate zone, can determine morphological changes of muscle fibres that include longitudinal stripes, contraction disks and torn fibres. Visually, these morphological changes were very similar to the contraction' knots of MTrPs described in pervious studies (Windisch et al., 1999, Simons and Stolov, 1976)

The available evidence indicates that MTrPs are a peripheral source of nociception (Shah et al., 2008, Shah et al., 2005, Arendt-Nielsen and Castaldo, 2015, Hsieh et al., 2012), where different types of endogenous substances like neuropeptides and inflammatory mediators, are implicated. Both hyperalgesia and allodynia have been observed at latent MTrPs indicating that MTrP can sensitize nociceptive and non-nociceptive nerve endings of muscles (Li et al., 2009). Moreover, an experimental investigation showed that an ischemic compression blockage of the large-diameter myelinated muscle afferents is associated with an increase of both pain pressure and referred pain thresholds at the MTrP' site (Wang et al., 2010). The same phenomenon was not observed at control sites, suggesting a role for non-nociceptive large-diameter myelinated muscle afferents in MTrP' symptomatology (Wang et al., 2010). These findings support the idea that the MTrP is characterized by a peripheral sensitization. Moreover, it has been

demonstrated that persistent nociceptive stimulation from MTrPs is associated with spatial pain propagation and that MTrP' inactivation can prevent widespread pain (Wang et al., 2012). The ongoing nociceptive afferent' activity can lead to abnormal function and structural changes in dorsal root ganglia and dorsal horn neurons (Camanho et al., 2011). In such conditions, an expansion of the receptive field of pain can occur (Shah et al., 2008) and patients can show hyperalgesia, allodynia and temporal summation of pain. Similarly, central sensitization can be postulated (Fernandez-de-las-Penas and Dommerholt, 2014). This hypothesis is supported by two studies showing that MTrP' stimulation alters the brain activity in regions processing stimulus intensity and pain affect (Niddam, 2009, Niddam et al., 2008). Specifically, a functional magnetic resonance study revealed an abnormal hippocampal hypoactivity that was attributed to a stress-related change due to the presence of MTrPs (Niddam et al., 2008). Central sensitization was also demonstrated in healthy subjects with mechanical hyperalgesia, within extra-segmental tissues after sustained mechanical stimulation of latent MTrPs (Xu et al., 2010). The complex interlinked pathophysiology described, which finally constitutes an expansion of the MTrP integrated hypothesis, is schematically outlined for an easier comprehension in figure 1.13.

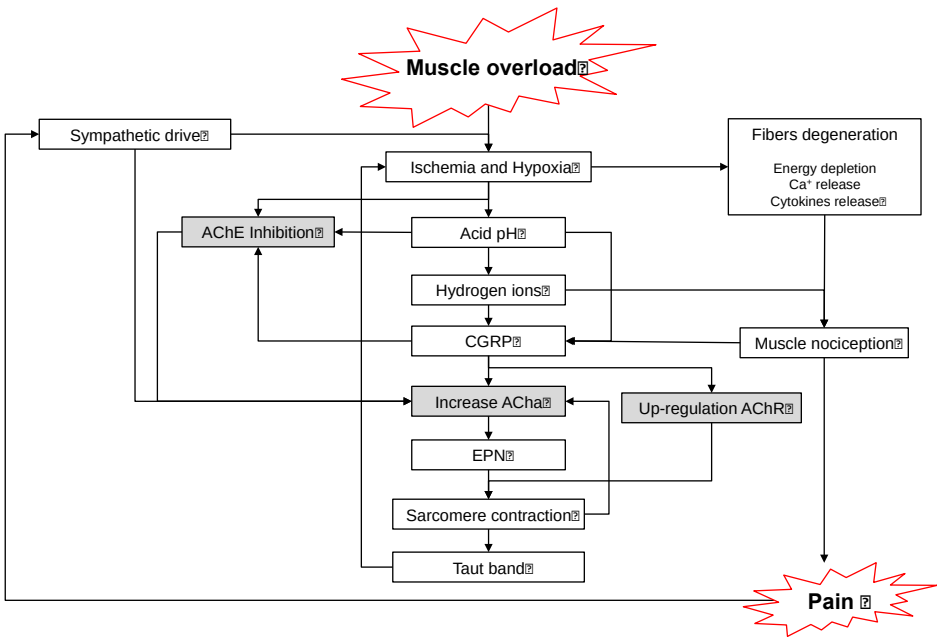

Figure 1.13.: Flow chart of the expanded trigger point hypothesis (Adapted from R. D. Gerwin, Dommerholt, & Shah, 2004).

1.7 DIAGNOSIS

Accurate history taking and physical examination are required in order to ensure a correct MPS' diagnosis. Initially, it is important to lead an anamnestic interview focusing on pain symptomatology. The key elements regarding pain, are: quality, intensity, timing, location and extent. Patients with MPS usually describe their pain using the following terms: dull, steady, deep or aching. A visual analogue scale should be provided to patients to measure pain intensity, at a daily or weekly frequency, and any correlation with activities of daily life must be recorded. Additionally, patients can use body charts to draw both pain' location and extent. A comparison with the MTrPs' reference maps will be helpful to hypothesize about which muscles have a MTrP. Nevertheless, as suggested by Simons in the second edition of his *Trigger point manual* (Simons et al., 1999), MTrP' referred pain maps should be considered nonspecific and constitute only a cue for clinicians. Physical examination, which is essentially a manual palpation protocol, is then performed to confirm the presence of the MTrP diagnostic criteria. Simons and Travell proposed the following minimal criteria:

1) Taut band palpable
2) Focal spot tenderness of a nodule in the taut band
3) Patient's recognition of pain complaint by manual compression of the spot tenderness (identifies an active MTrP)
4) Pain with stretching or contraction of the affected muscle

Another two additional confirmatory signs (not mandatory) are also proposed:

1) Local twitch response
2) Referred pain with expected distribution (Travell and Simons, 1983)

The minimal diagnostic criteria must be verified in the order proposed above, with manual palpation performed by drawing the fingertips of the examining hand forward and back, perpendicular to the muscle fibres (flat palpation technique) (figure 1.15). Alternatively, some muscle can be palpated between fingers and thumb using a pincer grip (figure. 1.15). Once the taut

band has been identified within the muscle, the spot tenderness should be located by gentle compression of contiguous spots along the taut band.

Figure 1.15: Manual palpation techniques. Left side, pincer grip palpation of the medial gastrocnemius muscle. Right side, flat palpation of the paraspinal muscles.

Beside palpation, clinicians must ask the following questions:

1) “Which of the following spots is the most painful?”
2) “Do you recognize this pain? Is it part of your usual complaints?”
3) “Does the pain refer anywhere from the spot that I am compressing?”

By definition, an affirmative reply to the first question will confirm the spot tenderness criteria. An affirmative reply to the second question will confirm the pain recognition criteria; and finally, the third question will confirm the referred pain’s presence. It is important to note that the physical examination of a subject with a possible MPS diagnosis, starts with the taut band investigation, which is, together with the local twitch response, the only objective sign among the MTrP criteria. All the other diagnostic criteria (i.e spot tenderness, pain recognition and referred pain) rely on pain elicitation, and therefore should be considered as subjective signs. To avoid misunderstanding, clinicians must provide patients with clear instructions and questions.

Finally, clinicians should consider different conditions other than MPS, that can potentially induce regional musculoskeletal pain. The following questions, proposed by Borg-Stein (Borg-Stein and Simons, 2002), may be useful in establishing the role of MTrP in generating the patient's painful symptomatology and establish any differential diagnosis:

. Is there regional myofascial pain, with MTrPs present?
. Is the myofascial pain the primary pain generator or are there other coexisting or underlying structural diagnoses?
. Is there a nutritional, metabolic, endocrinologic, psychologic, or inflammatory disorder that may be contributing to the regional pain?
. Is there widespread pain and other associated symptoms?

Specifically, the MPS' differential diagnosis should consider the following disorders (Cummings and Baldry, 2007):

- Joint disorders: zygapophyseal joint disorder, osteoarthritis, loss of normal joint range of motion.
- Inflammatory disorders: polymyositis, polymyalgia rheumatica, rheumatoid arthritis.
- Neurologic disorders: radiculopathy, entrapment neuropathy, metabolic myopathy.
- Regional soft tissue disorders: bursitis, epicondylitis, tendinitis, and cumulative trauma.
- Discogenic disorders: degenerative disk disease, annular tears, protrusion, and herniation.
- Visceral referred pain: gastrointestinal, cardiac, pulmonary, and renal.
- Endocrine: hypothyroidism.
- Psychologic disorders: depression, anxiety, and disordered sleep.
- Fibromyalgia or widespread chronic pain.

The MPS' diagnostic criteria described above are the only ones available, and although there is variability in their application, they are accepted by researchers and clinicians (Tough et al. 2007).

1.8 RELIABILITY OF DIAGNOSTIC CRITERIA

An adequate treatment is possible only with an accurate diagnosis. In the case of incorrect diagnosis, the treatment approach may be not appropriate. Thus reliability and validity are critical in the development of any treatment approach and should be addressed prior to any clinical trials. Travell and Simons originally proposed diagnostic criteria for the MTrP diagnosis. The validity of the MPS' diagnosis were considered reasonable and supported by some electrophysiological studies (Hong and Simons, 1998), while all the investigations depending on imaging techniques (Gerwin and Duranleau, 1997), or biopsy and electromyography, were not accepted as a reference standard for the diagnosis. A general agreement on diagnostic criteria among clinicians has not been reached, although clinicians recognised the clinical entity of MTrPs and researcher-led studies on the effectiveness of MTrP' treatments.

In 2007, Elizabeth Tough and her collaborators led a systematic review on diagnostic criteria for MTrP (Tough et al., 2007). The purpose of the study was to investigate the criteria used by expert clinicians to diagnose MPS. Specifically, they were interested in the frequency and the combinations of different MTrP' diagnostic criteria applied by researchers during their investigations. They selected 93 scientific papers including clinical intervention trials, diagnostic studies, and epidemiologic studies. The most frequently used criteria was "Tender spot in a taut band", which had been explicitly reported or implied in 65% of the selected papers. "Patient pain recognition on tender spot palpation" and "Predicted pain referral pattern" were identified in 53% of the papers. As expected, to diagnose MPS, a combination of specific criteria was usually applied. Notably the combinations

of specific criteria used were inconsistent and additionally, fifteen studies indicated only one criterion. Publications by Travell and Simons were cited as the main authoritative references, although their recommendations regarding the diagnostic criteria were not always followed correctly. The observed lack of a consistent approach in MTrP' diagnosis was probably due to the poor results reported in studies that investigated the reliability of manual palpation for MTrP' examination.

Several clinical studies on reproducibility of the diagnostic criteria for MTrP have been conducted and published in peer-reviewed journals between 1992 and 2005 (table 1.5) (Sciotti et al., 2001, Njoo and Van der Does, 1994, Nice et al., 1992, Lew et al., 1997, Levoska et al., 1993, Hsieh et al., 2000, Gerwin et al., 1997, Bron et al., 2007, Al-Shenqiti and Oldham, 2005). The study characteristics were different and included different populations, examiners, setting, muscles and variations regarding the diagnostic protocol.

Table 1.5: Intra- and inter-examiner studies on MTrP manual palpation.

Authors	Title	Year	Journal
Nice, et al.	Intertester reliability of judgments of the presence of trigger points in patients with low back pain	1992	Arch Phys Med Rehabil
Levoska, et al.	Repeatability of measurement of tenderness in the neck-shoulder region by a dolorimeter and manual palpation	1993	Clin J Pain
Njoo, et al.	The occurrence and inter-rater reliability of myofascial trigger points in the quadratus lumborum and gluteus medius: a prospective study in non-specific low back pain patients and controls in general practice	1994	Pain
Gerwin, et al.	Interrater reliability in myofascial trigger point examination	1997	Pain
Lew, et al.	Inter-therapist reliability in locating latent myofascial trigger points using palpation	1997	Man Ther
McKenzie, et al.	Prevalence of muscle trigger points in children with celebral palsy	1997	Phy Occup Ther Pediatr
Hsieh, et al.	Interexaminer reliability of the palpation of trigger points in the trunk and lower limb muscles	2000	Arch Phys Med Rehabil
Sciotti, et al.	Clinical precision of myofascial trigger point location in the trapezius muscle	2001	Pain
Bron, et al.	Interrater reliability of palpation of myofascial trigger points in three shoulder muscles	2007	J Man Manip Ther
Al-shenqiti, et al.	Test-retest reliability of myofascial trigger point detection in patients with rotator cuff tendonitis	2005	Clin Rehabil

Notably, two systematic reviews have been conducted with the aim of determining the reliability of physical examination for MTrP (Lucas et al., 2009, Myburgh et al., 2008). Both the reviews screened the literature using the most relevant electronic database and selected the studies using similar inclusion and exclusion criteria. Lucas and collaborators included 8 different studies, while Myburgh and collaborators only 6. All the selected studies investigated the reproducibility of manual palpation for MTrP identification using an appropriate repeated measures design, although several methodological biases were identified. The two reviews stated that an acceptable reliability was demonstrated only in a few studies and exclusively for some MTrP' diagnostic criteria. The k values reported, varied widely, and ranged from excellent to less than chance for each diagnostic criterion and for each muscle. Reliability was higher for subjective criteria like spot tenderness and pain reproduction, and definitely lower for taut band and local twitch response. Spot tenderness criteria for upper trapezius, and pain reproduction criteria for gluteus medius and quadratus lumborum showed the highest K values. Considering the lack of evidence for the MPS diagnosis, the authors recommended extreme caution in considering the clinical entity of MTrP. Any treatment based primarily on the MTrPs, can be misleading and may not lead to the best treatment available (Lucas et al., 2009).

A few important considerations can be drawn from the two systematic reviews. First, it is clearly indicated that the reliability of each MTrP criteria depends on the muscle being considered. For example, in muscle that lies deep, for any manual examination aimed to identify the taut band, the local twitch response and the spot tenderness will be unfeasible. Secondly, when the MTrP has a high irritability (i.e. low pain pressure threshold) and manifests the referred pain phenomenon, its examination is most reproducible. Again, training for examiners and a rigorous standardization of the MTrP' manual examination are fundamental for any future investigation. Finally, Myburgh suggested moving the MTrP examination from an individual criteria approach to a global assessment approach (Myburgh et al., 2011).

Gerwin adopted this approach and demonstrated a good reproducibility for the MTrP' diagnosis in a few muscles (infraspinatus, latissimus dorsi, sternocleidomastoid, trapezius, extensor digitorum) (Gerwin et al., 1997). In the global assessment, clinicians still consider the criteria originally proposed by Simons and Travell for the MTrP, but provide only an answer of whether or not a MTrP is present. This is in line with Tough et al. who reported in their review, that the number of MTrP criteria considered over the years has been progressively reduced (Tough et al., 2007). Indeed, the criteria not considered essential by the authorities have been abandoned and the diagnostic procedures refined over time. For example, predicted referral pain is now consider nonspecific and the local twitch response is no longer considered essential for the MPS' diagnosis (Gerwin 1997, Lewis 1999).

1.9 TREATMENT

Effective treatment of a musculoskeletal disorder requires accurate identification of the pain source and correct management of perpetuating factors. The MPS is a clear example of a painful disorder where any proposed treatment implies a precise localization of the pain generator (i.e. MTrP), but also a coping strategy for the perpetuating factors. Specifically, authors suggest that any mechanical or stressing events should be considered. Mense and Gerwin provide a comprehensive description of perpetuating factors in their textbook (table 1.6) (Mense and Gerwin, 2010). Clinicians generally overlook them, reducing the efficacy of their treatment (Fricton, 1991, Fricton, 1989). In some patients, the complexity of perpetuating factors requires a multidisciplinary approach. Is it also important to point out, as confirmed by electromyographic investigations, that any emotional or mental stress may activate or perpetuate a MTrP (Hubbard and Berkoff, 1993, Celik and Kaya Mutlu, 2012).

Various methods of MTrP treatment are available but currently, no clinical guidelines are available and clinicians are required to balance the evidence, their clinical experience and the patient's preferences.

Treatment approaches can be considered as invasive and non-invasive (Mense and Gerwin, 2010, Rickards, 2006). Dry needling or intramuscular stimulation, is an invasive technique in which a filiform needle is used to penetrate the skin and stimulate the MTrP (Vulfsons et al., 2012). The expected therapeutic effect is to release the taut band and reduce the irritability of the spot tenderness (Chou et al., 2012). Together with injections (local anaesthetics, steroids, Botulinum toxin A), these are among the most common treatments for MPS. Non-invasive treatments include various manual techniques and modalities (Mense and Gerwin, 2010, Rickards, 2006).

Recently dry needling gained popularity among physiotherapists and four systematic reviews on the efficacy have been completed. Tough and collaborators led a literature search, selecting studies where at least one group of patients were treated by dry needling into the MTrP and where a control group were enrolled (Tough et al., 2009). Pain measured using the VAS was considered to be the principal outcome. A meta-analysis was conducted using 4 out 7 selected studies, and dry needling was not found to be significantly superior to sham treatments (standardised mean difference, 14,9 [95%CI, -5,81 to 33.99]) (Itoh et al., 2007, Itoh et al., 2006, Ilbuldu et al., 2004, Huguenin et al., 2005). Among the studies excluded from the meta-analysis, one affirmed that dry needling was superior to no intervention (DiLorenzo et al., 2004), while the other comparing MTrP dry needling versus aspecific muscle needling (i.e. not in the MTrP), showed inconsistent results (Itoh et al., 2004, Chu, 1997).

Table 1.6: Perpetuating factors divided into three main categories.

Ergonomic factors	Structural factors	Medical factors
Work related activities	Scoliosis	Hormonal
Prolonged static postures	Leg-length inequality	hypothyroidism
Repetitive activites	Pelvis asymmetry	testosterone deficiency
Recreational activites	Hyper-mobility	nutritional
Telephone use	Hypo-mobility	iron deficiency
Computer use	Forward neck posture	Vitamin D deficiency

A more specific systematic review, aimed at verifying the efficacy of dry needling of MTrPs in patients with plantar heel pan, was completed in 2010 (Cotchett et al., 2010). Only three quasi-experimental trials, each with a low quality score were included in the review. The investigators concluded that there is limited evidence to support the treatment of plantar heel pain using MTrPs' dry needling. A systematic review with meta-analysis was able to support the use of MTrPs' dry needling in clinical practice. It was published in the Journal of Orthopaedic and Sports Physical Therapy and aimed to explore the evidence for MTrPs' dry needling in patients with MPS of the upper quarter (Kietrys et al., 2013). Twelve studies met the inclusion criteria and their quality score, according to the MacDermid Quality Checklist (scale range 0 to 48; maximal score 48), ranged from 23 to 40 points. Four different meta-analysis were completed: 1) immediate effect of dry needling versus sham or control, 2) efficacy at 4 weeks of dry needling versus sham or control, 3) immediate effect of dry needling versus a comparison treatment, 4) efficacy at 4 weeks of dry needling versus a comparison treatment. With regards to the results of 3 randomized clinical trials, the authors provide a grade A recommendation for dry needling for immediate pain relief, when compared to placebo, in the selected population (Hsieh et al., 2007, Tsai et al., 2010, Tekin et al., 2013). The effect size ranged from 1.2 to 4.9 points for the VAS score (maximum score, 10). Additionally, the results of two trials provided evidence that dry needling, in patients with upper quadrant MPS, can reduce pain at 4 weeks (Tekin et al., 2013, Itoh et al., 2007). It has to be noted that the overall effect was limited by a large confidence interval. Furthermore, many heterogeneous studies investigated the effect of dry needling in comparison to a variety of different treatments, with results ranging from no difference, to a difference favouring either dry needling or the comparison treatment (Cotchett et al., 2010). However, a recent systematic review without meta-analysis, on dry needling for MTrP in the upper trapezius muscle, found strong evidence on pain reduction (Cagnie et al., 2013). Eight clinical studies with low quality were examined (Hong, 1994a, Itoh et al., 2007, Ma et al., 2010, Myburgh et al., 2012, Ay et al.,

2010, Eroglu et al., 2013, Ga et al., 2007a, Ga et al., 2007b). Also, this review, in agreement with Kietrys (2013), declared the need for a high-quality study design to provide more robust evidence on the dry needling' efficacy.

Many different manual therapy techniques to treat and manage MPS have been investigated in controlled studies: ischemic compression (Kim et al., 2013, Martin-Pintado-Zugasti et al., 2015, Montenegro et al., 2015); MTrP' pressure release (Montenegro et al., 2015, Grieve et al., 2011, Grieve et al., 2013b); myofascial induction technique (Saiz-Llamosas et al 2009); passive stretching (Hanten et al., 2000); muscle energy techniques (Yeganeh Lari et al., 2016, Oliveira-Campelo et al., 2013); strain counterstrain (Segura-Ortí et a 2016, Wong CK et al 20014, Ibáñez-García J et al 2009); and, high velocity low amplitude thrust (Srbely JZ et al 2013, Ruiz-Sáez et al 2007). In addition, many studies have been conducted for different modalities, such as ultrasound (Kavadar G et al 2015, Benjaboonyanupap D et al. Manca A et al 2014, Kim Y et al. 2014), low level laser (Demirkol N et al 2015, Manca A et al 2014, Gur A et al 2004, Hakgüder A et al 2003) and transcutaneous electrical nerve stimulation (Gemmell H et al 2011, Rodríguez-Fernández AL et al 2011, Graff-Radford SB et al 1989, Salim M. et al 1992).

Fernandez de las Penas (2005) selected for a systematic review, 7 trials that included at least one group receiving a manual therapy treatment (Fernandez-de-Las-Penas et al., 2005). Selection of the studies was limited by the lack of uniformity regarding the outcome measures and by the poor interval validity. Only 2 of the selected studies included a visual analogic scale, PPT and range of motion (Hou et al., 2002, Hanten et al., 2000); the remaining studies reported only a limited number of outcomes (Hanten et al., 1997, Hong et al., 1993, Jaeger and Reeves, 1986) or selected non appropriate outcome measures such as tenderness or posture (Gam et al., 1998, Dardzinski et al., 2000). Moreover, the PEDro score for internal validity reached 6 out of 10 only in two studies (Gam et al., 1998, Hong et al., 1993), and in 3 studies, was less than 4 out of 10 (Jaeger and Reeves, 1986,

Dardzinski et al., 2000, Hanten et al., 1997). Also, 4 of the selected studies included only one treatment session (Jaeger and Reeves, 1986, Hou et al., 2002, Hong et al., 1993, Hanten et al., 1997).

The review's findings did not demonstrate any evidence in favour of manual therapy techniques, even when associated with other treatments such as ultrasound and massage. The hypothesis that manual therapy for MTrP has a specific efficacy beyond placebo, was neither supported nor refuted. Nevertheless, some trials included in the review confirmed that MTrP' treatment might reduce the pressure pain threshold of spot tenderness and the VAS score (Fernandez-de-Las-Penas et al., 2005). Notably, the author remarked upon the need to assess ROM in future clinical trials, as it is an important outcome measure in patients with MTrP.

The efficacy of non-invasive treatments has been investigated also by Rickards (2006). The author conducted a systematic review including 23 controlled or quasi-randomized trials. Only studies which clearly stated the MTrP diagnostic criteria and with concealed allocation, were included in the review. The selected trials were divided into 5 categories and the following treatments considered: laser therapies, electrotherapies, ultrasound, magnet therapies, and manual therapies. A short-term efficacy was confirmed for decreasing MTrP' pain intensity by laser therapy and transcutaneous electrical nerve stimulation (Gur et al., 2004, Snyder-Mackler et al., 1989, Hakguder et al., 2003, Ilbuldu et al., 2004, Ceccherelli et al., 1989, Graff-Radford et al., 1989, Farina et al., 2004, Hsueh et al., 1997). Moderate evidence supporting ultrasound was available from one high quality and two lower quality clinical studies, suggesting that ultrasound is not an effective MTrP' treatment (Lee et al., 1997, Gam et al., 1998, Esenyel et al., 2000). Again, moderate evidence was also confirmed for magnet therapies (Smania et al., 2005, Smania et al., 2003, Brown et al., 2002). Notably, 4 high quality studies on manual

therapy have been identified in which short-term efficacy was confirmed (Hanten et al., 2000, Gam et al., 1998, Chatchawan et al., 2005, Fernandez-de-Las-Penas et al., 2006b). Overall, the evidence for effectiveness of the different non-invasive MTrP' treatments was based on a limited number of studies and in a few cases, significant methodological biases were identified (i.e. unblinded assessors, low quality scoring system).

In 2015, an original systematic review on the use of ischemic compression and dry needling for MTrPs was completed (Cagnie et al., 2013). Fifteen studies were selected, 80% were scored as level B studies (Ay et al., 2010, Eroglu et al., 2013, Ga et al., 2007a, Ga et al., 2007b, Ma et al., 2010, Aguilera et al., 2009, Hanten et al., 2000, Kannan, 2012, Oliveira-Campelo et al., 2013, Nagrale et al., 2010, Fernandez-de-Las-Penas et al., 2006b, Gemmell and Allen, 2008), and the remaining 20% were at level A2 (Hong, 1994a, Itoh et al., 2007, Myburgh et al., 2012). Notably, the blinding of the operator and/or the patients was identified as a major limitation. For all the selected studies, only short and medium term outcomes were considered. Seven studies investigated the efficacy of the ischemic compression and 8 the efficacy of dry needling. Findings were in line with those reported in the previous reviews: moderate evidence was found in favour of ischemic compression and strong evidence was found for dry needling. According to the available systematic reviews dry needling should be considered the first choice treatment but due to the small number of high quality trials additional research requires to be undertaken.

1.10 RESEARCH AGENDA.

Myofascial pain research has increased since the publication of the first edition of "Travell and Simons' Myofascial Pain and Dysfunction: Trigger Point Manual" (Travell and Simons, 1983) and many researchers are

currently leading both pure and clinical research. Simons, Mense, Gerwin and Hong did an extraordinary amount of work to address the MTrP enigma, and laid the foundations for further studies. It is now important to define priorities and develop a research agenda. In order to fulfill this aspiration a number of researchers have proposed areas for further MTrP research (Simons, 2004, Mense and Gerwin, 2010, Dommerholt and Huijbregts, 2011).

1.10.1 Aetiology

Many theories regarding the development of MTrPs have been proposed but there is a lack of an officially recognised aetiology. It is postulated that a taut band and a latent MTrP are the first abnormalities, and that the active MTrP and the referred pain, are secondary stages of the MPS' clinical course (Dommerholt and Huijbregts, 2011). It is not clear what the specific causes or risk factors are that trigger this sequence of events. Similarly, what is the relationship between active and latent MTrPs should be clarified. Finally, although evidence of endplate dysfunction in muscle harbouring a MTrP is available, the relationship between the MTrP and the endplate dysfunction should be further investigated. The integrated hypothesis (Simons et al., 1999, Gerwin et al., 2004) defines the MTrP as a neuromuscular condition characterized by two components: a sensory locus and motor locus (Kuan, 2009).

The sensory component has been defined as the muscle site where pain (i.e. hyperalgesia and allodynia), referred pain, and a local twitch response can be evoked by manual compression or dry needling (Dommerholt et al., 2006). Referring to the manual palpation protocol, it is essentially the spot tenderness located within the taut band of muscle (see diagnostic criteria in Chapter 1). The motor component has been defined as the muscle site where the SEA can be recorded using needle EMG, and according to the integrated hypothesis, this is an electromyographic signal that arises from dysfunctional endplates.

According to this definition of a MTrP and to the integrated hypothesis, it is reasonable to assume that the MTrP spot tenderness (i.e. the sensory locus) and the endplates are located in the same region, and are at least partially overlapped. Although no research studies have been conducted on this specific issue, authors indicate a close spatial relationship between the MTrP and the endplate region. Simons in 2002, during an investigation on the prevalence of the motor endplate potentials (i.e. SEA) in active MTrP, examined 11 muscles of 10 subjects using needle EMG. The SEA was recorded and detected within the MTrP region and also close to this, within the endplate zone (Simons et al., 2002). This zone is also known as the innervation zone (IZ) in the literature (Buchthal and Rosenfalck, 1966). Buchthal & Rosenfalck published these results in this American Journal of Physical Medicne & Rehabilitation and clearly stated: "*In this study, the MTrPs were consistently found within an endplate zone*." Later, Kuan (Kuan, 2009) corroborated this finding in a literature review: "*In skeletal muscle, a tender point can be an myofascial trigger point if it locates in the endplate zone with all characteristics of an MTrP, such as taut band, referred pain, and local twitch response"*.

The SEA is currently accepted to be an electromyographic signal that arises from a group of dysfunctional endplates and in the current literature, is named endplate noise (Simons, 2001, Simons et al., 2002, Kuan et al., 2002, Hong, 2002, Chou et al., 2009, Gerwin et al., 2004). The reported association between the endplate noise and the MTrP has led to the suggestion that the MTrP region is where the endplate zone is located (Kuan et al., 2007, Mense and Gerwin, 2010).

Nevertheless, it is important to note that dysfunctional endplates in MTrP have never been demonstrated, and the accuracy of needle EMG in detecting and locating the IZ, has never been explored.

As affirmed by Mense (2001) in his book on muscle pain:

"*Understanding the location of motor endplates is important for the clinical diagnosis and management of myofascial trigger points. Since the*

pathophysiology of the myofascial trigger point is intimately associated with endplates, one expects to find TrPs only where are motor endplates."

1.10.2 Diagnostic gold standard

Current diagnosis of MTrP relies on history taking and physical examination of patients, and strongly depends on clinical skill and experience. A few diagnostic procedures like electromyography (Simons et al., 2002), thermography (Wolf, 1989, Diakow, 1992), skin resistance, ultrasound (Gerwin and Duranleau, 1997), have been proposed, but none of these has been accepted as a diagnostic gold standard. A growing body of evidence indicates taut bands can be visualized during ultrasound-guided examination, especially using sonoelastography or multidimensional imaging (Thomas and Shankar, 2013, Shankar and Reddy, 2012, Sikdar et al., 2008). Using mechanical vibration during sonoelastography, Sikdar (2008) demonstrated abnormalities of muscle containing MTrPs. They described a nodular region characterized by hypoechogenicity. The examined MTrPs showed a diminished vibration amplitude consistent with the taut band site (Sikdar et al., 2008). It was suggested that active MTrPs are not necessarily associated with isolated nodular lesions, but that active MTrPs are associated with heterogeneity of the muscle (Ballyns et al., 2011, Sikdar et al., 2010). Forty-four patients with acute cervical pain and one active MTrP were examined using sonoelastography, and the authors distinguished normal muscle from active MTrPs (Ballyns et al., 2011). Notably, no correlation between active MTrPs and the PPT was found, and the data regarding the MTrP size was not clearly reported. Moreover, a control group was not included in the study. However and notably, no correlation between an active MTrP and the PPT was found and the data regarding the MTrP size was not clearly reported. Moreover, a control group was not included in the study. However, the latest findings with ultrasound vibration elastography, confirmed the previous findings, and demonstrated how an effective dry needling treatment can

change the muscle tissue properties (Turo et al., 2015). These findings provide an important insight into the treatment of MTrPs, but it is important to note that the proposed ultrasound technique has never been fully validated, and its capability to assess the MTrP' tissue properties requires further research. A preliminary attempt to identify and quantify the MTrP' taut band using magnetic resonance elastography, has been undertaken on two subjects with chronic myofascial pain (Chen et al., 2008, Chen et al., 2007). The reported findings suggested that stiffness of the taut bands, in patients with myofascial pain, may be 50% greater than that of the surrounding muscle tissue. The authors did not provide any details about the diagnostic criteria for MTrP and did not describe the location of MTrP' spot tenderness with respect to the taut band. The same author concluded a study using the same magnetic resonance imaging technique on a convenience sample of 65 subjects with myofascial pain (Chen et al., 2016). The intra- and inter-reliabilities of the magnetic resonance imaging was excellent and the presence of the taut bands in upper trapezius confirmed. Unfortunately, the authors didn't locate the position of the MTrP' spot tenderness with respect to the taut band. Nevertheless, the agreement between physicians and the magnetic resonance imaging findings, was relatively poor. The taut band mean stiffness was 11.5 kPa while in the control site was 5.8 KPa. The authors asserted that clinicians may overestimate the presence of the taut band, while the magnetic resonance imaging may underestimate its presence (Chen et al., 2016). These imaging techniques are promising and may assume an important role in the management of MPS. Future studies need to resolve which imaging techniques have the highest accuracy in detecting MTrPs, and which have the greatest applicability in clinical practice.

1.10.3 Treatment

It appears that MTrP inactivation can be achieved using different treatment approaches. According to the available evidence, invasive treatments such as dry needling or substances injection, are considered more effective than non-invasive ones (Cagnie et al., 2013, Kietrys et al., 2013, Cotchett et al.,

2010, Fernandez-de-Las-Penas et al., 2005, Rickards, 2006, Tough et al., 2009). The efficacy of manual techniques or modalities has been explored in clinical trials with poor internal validity (Fernandez-de-Las-Penas et al., 2005, Rickards, 2006, Cagnie et al., 2013). Thus, high quality randomized clinical trials on the efficacy of non-invasive treatment are required. Moreover, it is important to define which manual therapy techniques are the most appropriate to treat MTrPs. Regarding this issue, Simons, after the publication of the motor endplate hypothesis (Simons et al., 1999), proposed an original manual approach named “trigger point pressure release”. The author suggested that the classic heavy ischaemic compression of MTrPs should be avoided, in order to avoid tissue hypoxia. As an alternative, he proposed a passive muscle lengthening until tissue resistance, with a slow and gentle MTrP compression. The hypothesis was that pressing and stretching the tissue uncouples myosin from actin in the MTrP region, a process that usually requires ATP. The described manual technique may also release the ”stuck” spring function of the titin connection to the Z bands of the sarcomeres (Dommerholt and Huijbregts, 2011). The theory described is very interesting, although based on anecdotal evidence, and needs basic physiological research to be confirmed.

Moreover, preliminary results on low-level laser therapy has created much interest among researchers (Uemoto et al., 2013). Considering the potential complications and limitations of invasive (Brady et al., 2014) techniques, the availability of an effective non-invasive treatment will be relevant for clinicians.

CHAPTER 2

RESEARCH QUESTION

2.1 - RESEARCH AIM, METHODOLOGICAL ASPECTS AND ETHICAL CONSIDERATIONS

The integrated hypothesis suggests that abnormal depolarization of the post-junction membrane of motor endplates induces a focal sarcomere contraction. This would induce a hypoxic energy crisis of the involved fibres, associated with sensory and autonomic reflex arcs (Bron and Dommerholt, 2012, Gerwin et al., 2004). The presence of the SEA at the endplate sites (Simons et al., 2002), and the clinical evidence that treating MTrPs significantly reduces the endplate noise, support the notion that MTrP are located in close proximity to dysfunctional motor endplates of the IZ (Ge et al., 2011, Kuan et al., 2007). The commonly encountered locations of MTrPs and their pain reference zones have been illustrated using standardized body charts, but there are no studies that describe the specific anatomical location of MTrPs.

The aim of this work will be to describe the location of MTrPs and the IZ in the upper trapezius muscle. An accurate description of the location of both the IZ and the MTrP will provide data to unravel their spatial relationship and to significantly contribute to the body of knowledge, associated with the MTrP. In order to plan the research activities, it was fundamental to establish:

- Which skeletal muscles might be best used to describe the MTrP' location?
- Which technique should be applied to optimally describe the IZ' location?

The upper trapezius muscle was selected for two main reasons. First, MTrPs in the upper trapezius are very common in subjects with mechanical neck pain, as well as in healthy subjects (Fernandez-de-las-Penas et al., 2007). A high prevalence of a MTrP was considered important to reduce the effort in the enrolment phase. Secondly, a higher reliability in the upper trapezius, for most of the diagnostic criteria, has been reported in studies on MTrP palpation procedures (Lucas et al., 2010a, Myburgh et al., 2008). Alternative approaches to manual palpation that establish the presence and

location of MTrPs, were not available. Moreover, the manual palpation procedures and diagnostic criteria proposed by Simons, are considered a reference standard (Simons et al., 1999, Tough et al., 2007). Again, the upper trapezius muscle morphology was considered suitable. Its surface is wide enough while its thickness is reduced and, it is reasonably practicable to describe the spatial distribution of both the MTrPs and the IZs. To select an appropriate technique to detect the IZ' location in the upper trapezius muscle, only non-invasive and painless procedures were considered. This approach was a priority due to ethical reasons, but also to avoid any increase of the MTrP' irritability. Needle EMG was excluded not only because it is an invasive tool, but also because the uptake area of a concentric needle electrode is approximately 2.5 mm (Merletti and Parker, 2004).

A description of the IZ' location, which is an anatomical structure and which involves a large portion of muscle, would be not feasible without sampling the EMG signals in many different regions of the upper trapezius. This approach, even though practicable, would give rise to many ethical concerns. Alternatively, the IZ' location can be identified from surface EMG signals, detected using linear arrays of electrodes, placed along the direction of the muscle fibres. In such a configuration, the IZ appears as the area from where the electrical potential propagates in two opposite directions towards the tendon' regions (Masuda et al., 1983b, Masuda et al., 1983a). This method has been originally proposed by Masuda and Sadoyama (1988), who also provided an appraisal of its validation. A number of studies have investigated the use of surface electrode arrays to locate the IZ within specific muscles (Falla et al., 2002, Iwasaki et al., 1990, Masuda et al., 1983b, Masuda et al., 1983a, Saitou et al., 2000). The IZ has been successfully identified in muscles having fibres running parallel to the skin (Saitou et al., 2000). This further supported the choice of the upper trapezius muscle as the experimental muscle. Indeed, it is composed of three different portions (i.e. upper, mid and lower), each with a different direction of fibres, but in which the fibres are always parallel to the skin surface (Johnson et al., 1994). Surface EMG showed a few other advantages when compared to

needle EMG. It is safe, and it is not necessary to penetrate the skin in order to record meaningful information regarding the motor units' action potentials. Surface EMG' application was considered an optimal technical solution to protect participants and to minimize their discomfort. Additionally, to generate EMG signals, it is enough to perform an isometric muscle contraction. Finally, it is technically easy to apply electrodes to the skin surface to detect EMG signals.

2.2 - RESEARCH HYPOTHESIS

As previously mentioned, the aim of this work was to describe the location of both the IZ and the MTrP in the upper trapezius muscle. The research design involved a cross-sectional study in which experimental data (i.e. IZ' and MTrP' location) was collected in a given population, at the same point in time (Mann, 2003). The intention was to describe the spatial relationship between them, while acknowledging that some authors have proposed that MTrPs are located in the IZ (Mense et al., 2001, Kuan, 2009, Ge et al., 2011). In other words, this means that theoretically, the distance between them should approximate to zero, or that they share the same location. Then the following null hypothesis (H_0) can be proposed:

. H_0: The distance between the IZ and the MTrP in upper trapezius muscle is equal to zero

The proposed hypothesis characterised the subjects with MTrP in the upper trapezius as the independent variables, and the IZ' location and the MTrP' location as the dependent variables. The statistical analyses involved an appropriate, parametric, or non-parametric, difference test, aimed at retaining or rejecting statistically that the distance between the two dependent variables (i.e. the IZ' location and the MTrP' location) was significantly different from zero.

Errors in the hypothesis testing must be considered in all research studies. Two types of errors are possible: type I (rejecting a null hypothesis when it is true) and type II (not rejecting a null hypothesis when it is false). In the case of type I error, the distance between the IZ' location and the MTrP' location is interpreted as being significantly different from zero, when in actual fact, it is zero. While in type II error, the distance between IZ and MTrP' locations is interpreted as having been not significantly different from zero, when it had been zero in actuality.

In the attempt to provide an answer to the research question, it was important to consider the risk for both the types of errors and to interpret the inferential statistic' results with caution. For this reason, an investigation to define the reliability (relative and absolute) of the procedures to locate the IZ and the MTrP will be useful for discussing the results of the cross-sectional study. Then the following additional null hypothesis can be proposed:

- H_0: The reliability of surface EMG in locating the IZ in upper trapezius muscle is lower than 0.60 (K value)
- H_0: The reliability of manual palpation in locating MTrP in upper trapezius muscle is lower than 0.60 (Intraclass correlation coefficient)

2.3 - RESEARCH PLANNING

An effective research planning includes the selection of an adequate research strategy for producing the necessary evidence to answer the research questions. The selection of the appropriate research strategy depends mainly on the research hypothesis. In the study, as it has been proposed, the extent of the reliability associated with the selected measurement procedures had been critical, as IZ' location and MTrP' location were the dependent variables. In this context, reliability reflects the reproducibility of a measurement procedure or tool.

As previously mentioned, the IZ' location in the upper trapezius muscle was inferred using measurements derived from surface EMG. Whereas, by contrast, the MTrP' location in the upper trapezius muscle was measured by a trained operator, using manual palpation. Thus, it was important to complete an investigation for both procedures to quantify both absolute and relative reliability (Safrit and Wood, 1989). Whereas relative reliability is the degree to which elements keep their position stable in a sample during repeated measurements. This kind of reliability is evaluated using correlation statistics. While absolute reliability is the degree to which repeated measurements change their values for the same elements (Safrit and Wood, 1989). In this case, reliability is reported using units of measurement or proportion of measured values (Bruton et al., 2000).

Single estimations of the reliability are not sufficient to define the reliability of a measurement procedure. The relative reliability of the measurement procedures will have to overcome some minimal correlation values. These values are reported in the literature and depend on the selected statistics (i.e. correlation coefficients, Kappa values, intraclass correlation coefficient) (Landis and Koch, 1977, Cohen, 1960, Munro, 2005). While absolute reliability will have to define the size and range of the measurement differences and these will be discussed in terms of clinical acceptability.

Findings of the cross-sectional study, aimed at describing IZ' and MTrP' locations, were to be discussed while considering the results of the two reliability studies. The thesis included two reliability studies and one conclusive cross-sectional study. An optimal planning of the mentioned studies would have been as follows: first, the study aimed at establishing the reliability of IZ' localization using surface EMG; second, the study aimed to establishing the reliability of MTrP' localization in the upper trapezius muscle using manual palpation; finally, the cross-sectional study (figure 2.1).

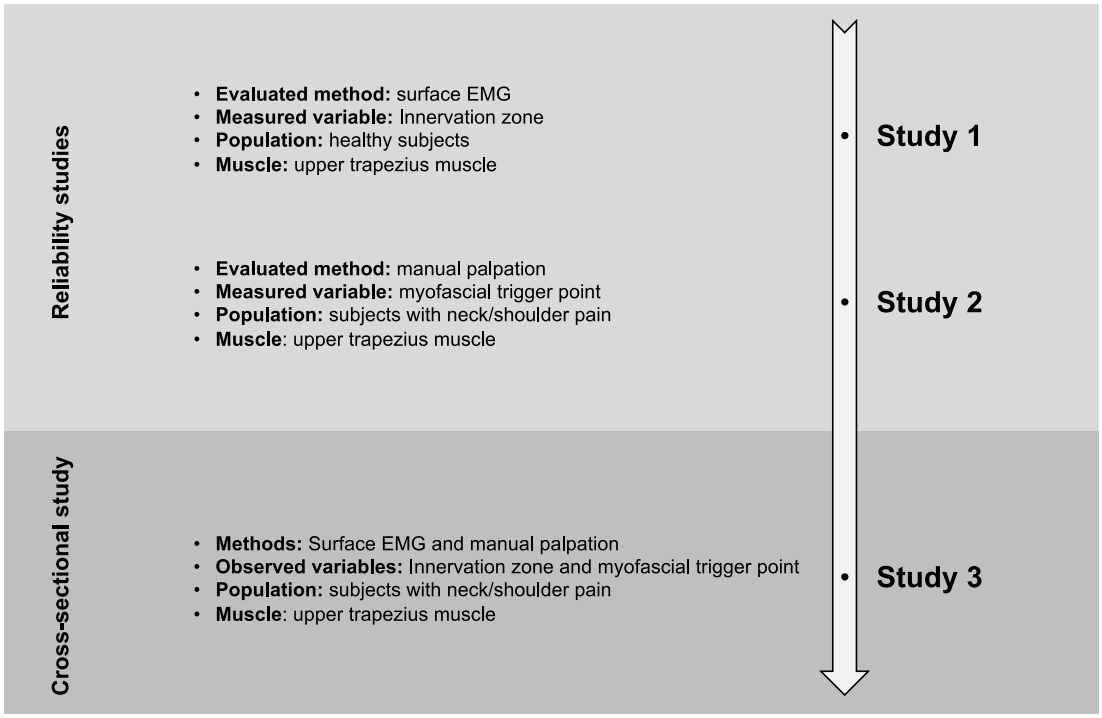

Figure 2.1: Research planning chart including main features of the studies.

2.4 – ACTUAL RESEARCH PLAN

As regards to the research planning, it is important to note that the study on reliability of manual palpation in locating the MTrP, had been conducted after having been delayed (i.e. conducted in 2011) and specifically after the cross-sectional study (i.e. completed in November 2008) (figure 2.2). The study on the reliability of surface EMG in locating the IZ had been conducted first, as planned. The implementation of the study on reliability of MTrP localization had been postponed necessarily due to the limited availability of the Laboratory of movement analysis of the Vita-Salute San Raffaele University (Milan, Italy). Research activities at the latter laboratory were prioritorised and supported according to an internal research agenda that wasn't controlled by the author. The deferral of the investigation on reliability of MTrP' localization should be considered an amendment to the original research planning that hadn't directly impacted on the proposed research project. Indeed, the research design and the experimental procedures of the

last study (i.e. the cross-sectional study) had not been constrained by the findings of the previous studies (i.e. the reliability studies). The selection of suitable outcome measures for use within the thesis had been extremely limited in practice (for the reasons mentioned previously). However, additional reliability-based clinometric criteria on which to appraise the selection of outcomes might have been compromised by the temporal re-organisation of the studies' execution. Findings of the reliability studies have been used for an evidence based discussion of the cross-sectional study on the spatial relationship between MTrP and IZ, as had been planned originally.

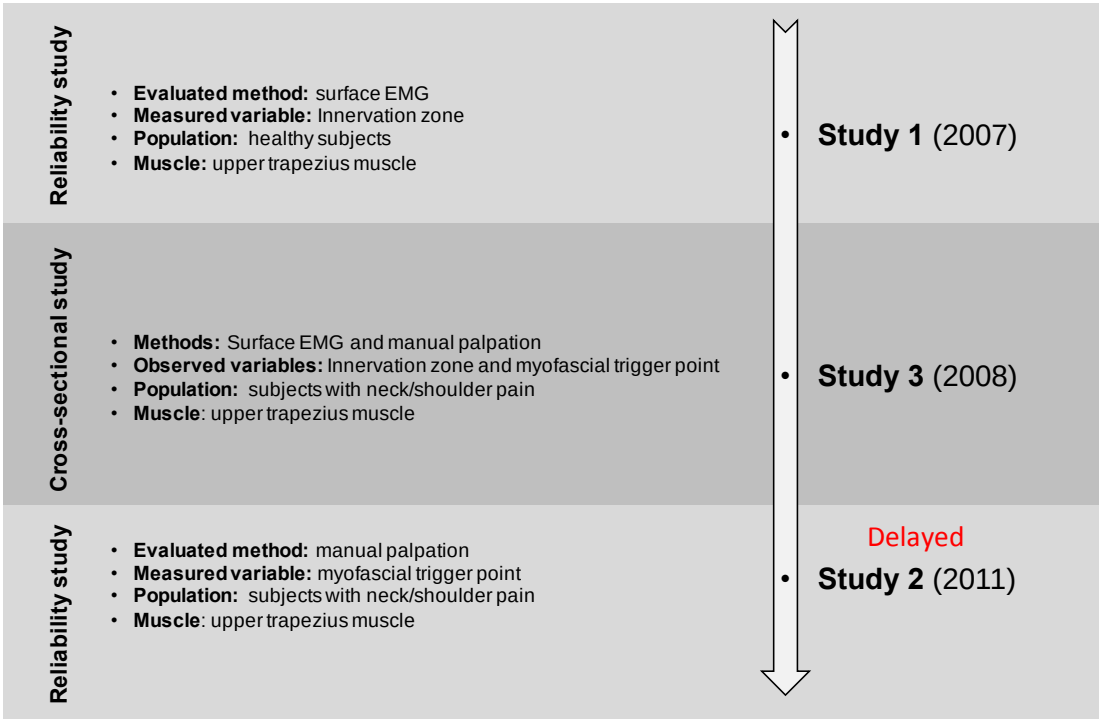

Figure 2.2: Chronological order of studies.

2.5 - RELEVANCE OF THE PROJECT

The relevance of a scientific investigation is gauged by the changes it introduces in the body of knowledge. In this thesis, the integrated hypothesis,

as suggested by current literature, had been accepted and the endplate dysfunction considered a central element of the MTrP's pathophysiology. Research studies supported the presence of an abnormal EMG pattern (i.e. SEA) within the MTrP's region. In spite of the criticisms raised by some experts, the abnormal EMG pattern has been attributed to the endplate and named endplate noise (Johnson, 2002). Moreover, the overlapping of the endplate noise and MTrP's spot tenderness that had been proposed (Mense, 1999, Simons et al., 2002, Kuan, 2009). Evidence that would support or refuse the overlapping spatially between the IZ and the MTrP will potentially provide insights into various fields of the MTrP research.

Primarily, the research findings will contribute to the development of understanding of the MTrP physiopathology. An overlapping geometrically between the IZ and the MTrP will indeed reinforce the conceptual model for endplate dysfunction underpinning the phenomenon of MTrP. Otherwise, investigations of the geometric location will open new research perspectives to elucidate the nature of MTrP' spot tenderness. The overlapping between IZ and MTrP will also support any therapy directed at muscle endplates (i.e. botox). Finally, if confirmed, the overlapping will also define the location of the MTrP according to an anatomical structure (i.e. IZ). Indeed, it is not clearly understood if a MTrP can be located anywhere within the belly of a muscle. The MTrP' charts proposed by Simons (Simons et al., 1999), based on a previous study led by Janet Travell and Seymour Rinzler (1952), indicate a few possible locations for the MTrPs within various muscles, but these offer only approximate locations. During manual palpation, operators examine the entire muscle belly to locate both the spot tenderness and the taut band. If confirmed, a MTrP' location anchored to the IZ, will facilitate the diagnostic procedure in several muscles, like for example, the bicep brachii muscle, where the IZ is constantly located in a well-defined region (mid portion of the belly) (Masuda et al., 1983a). Thus, in this muscle, it will be possible to reduce false positive findings by distinguishing nonspecific

hyperalgesia (i.e that can be located theoretically in any region of the muscle belly) and a MTrP.

CHAPTER 3

RELIABILITY OF SURFACE EMG MATRIX IN LOCATING THE INNERVATION ZONE OF UPPER TRAPEZIUS MUSCLE

3.1 SUMMARY

The identification of the motor unit IZ using surface EMG signals detected on the skin with a linear array or a matrix of electrodes has been recently proposed in the literature. However, an analysis of the reliability of this procedure and, therefore, of the suitability of the surface EMG signals for this purpose has not been reported.

The purpose of this work is to describe the intra and inter-rater reliability and the suitability of surface EMG in locating the innervation zone of the upper trapezius muscle.

Two operators were trained on electrode matrix positioning and sEMG signal analysis. Ten healthy subjects, instructed to perform a series of isometric contractions of the upper trapezius muscle participated in the study. The two operators collected sEMG signals and then independently estimated the IZ' location through visual analysis.

Results showed an almost perfect agreement for intra-rater and inter-rater reliability. The constancy of IZ' location could be affected by the factors reflecting the population of active motor units and their IZs, including: the contraction intensity, the acquisition period analysed, the contraction repetition. In almost all cases the IZ' location' shift due to these factors did not exceed 4 mm. Results generalization to other muscles should be made with caution.

3.2 INTRODUCTION

The motor unit is considered to be the basic functional unit of the neuromuscular system; it is composed of a group of muscle fibres and the somatic motor neuron. When the somatic neuron fires an action potential, all fibres included in the motor unit contract, converting an electrical stimulus to a mechanical response (Standring and Gray, 2008). The motor unit's action potentials travel along the fibres of that motor unit with a conduction velocity ranging from 2.6 to 5.3 m/s (Andreassen and Arendt-Nielsen, 1987).

Differently from the autonomic pathways (i.e. parasympathetic pathway, sympathetic pathways, and adrenal sympathetic pathway), the somatic motor pathways contain a single neuron. As with the other types of synapses, the neuromuscular junction includes three main components: 1) the motor neuron's presynaptic axon terminal, that contains synaptic vesicles and mitochondria; 2) the synaptic cleft, that is a gap of approximately 30 nm between the presynaptic axon and the postsynaptic membrane; 3) the postsynaptic membrane of the muscle fibres (i.e. the motor end plate), that contains high concentrations of nicotinic acetylcholine receptors (Marieb and Hoehn, 2013). The synaptic cleft contains acetylcholinesterase, an enzyme that breaks down the neurotransmitter acetylcholine. The cleft is filled with a fibrous matrix containing collagen fibres that bind the axons of a motor nerve with the muscle fibres (figure 3.1) (Sine, 2012).

The IZ (i.e. the motor endplate region) of a muscle is the site where axons of the motor nerve divide into a number of branches that end in a claw-like motor endplate. The IZ of a muscle has been described in the literature as a narrow band usually running perpendicular to the middle of the muscle fibres (Defreitas et al., 2008, Mense et al., 2001). Coërs and Woolf (1959) were the first authors to describe this principle in human, combing muscle biopsies and intravital staining with methylene-blue (Coërs and Woolf, 1959). The

same spatial arrangement of the IZ was also reported in several muscles of stillborn infants (Christensen, 1959).

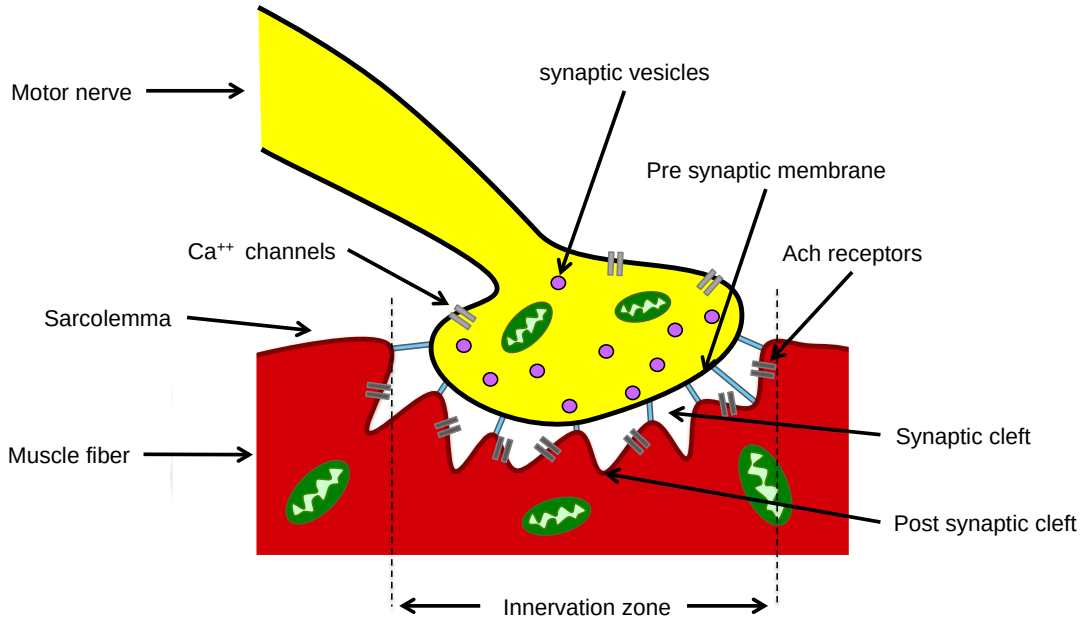

Figure 3.1: Neuromuscular junction and the innervation zone.

The morphology of the IZ depends on the fibres' orientation and thus, muscle architecture is important in understanding the IZ' location within muscles, and therefore as previously debated, to estimate the possible MTrP' locations. Fibre' architecture falls into two major categories, parallel and pennate (Fukunaga et al., 1997). In parallel muscle, fibres are parallel to the length of the muscle and the IZ' morphology can be described as a straight line, perpendicular to midfibres. Examples of this architecture type are the biceps brachii muscle and sternocleidomastoid muscle. Differently, pennate muscles have one or more tendons that extent over the length of the muscle, and their fibres run obliquely to insert into tendons (Oatis, 2009). The IZ' morphology in these muscles is an irregular curved line: upper trapezius muscle and deltoid muscle are two examples (figure 3.2). Nevertheless, the

principle of using a midfibres region for identifying the IZ' location, is not always applicable.

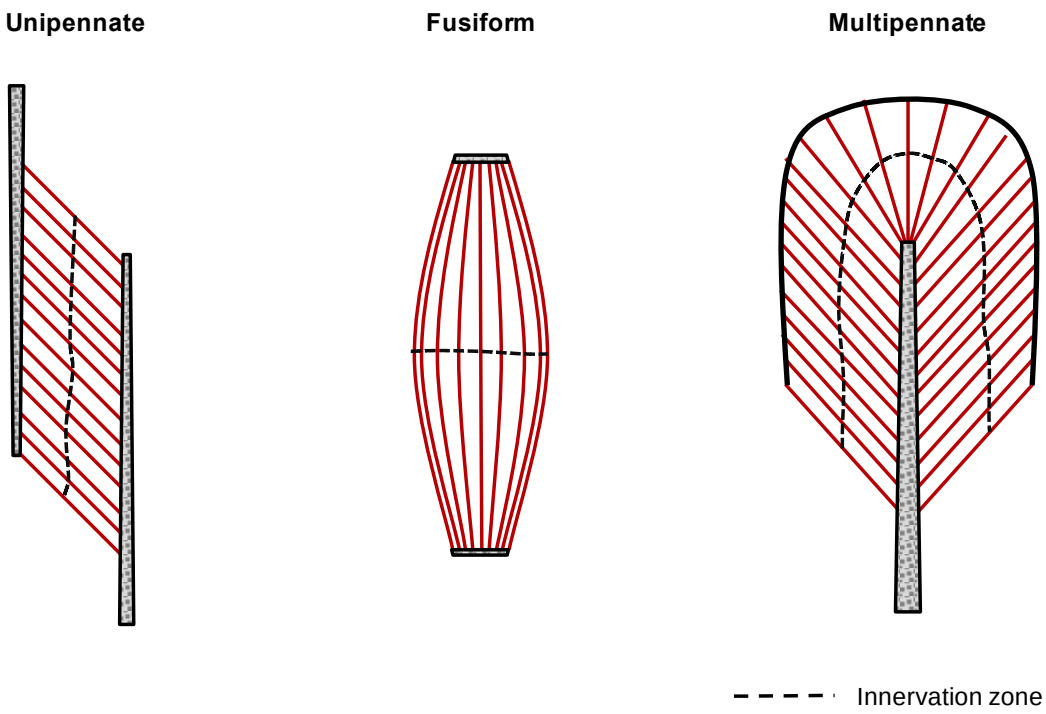

Figure 3.2: Location of the innervation zone in human skeletal muscles with different architectures (Adapted from Mense, 1999)

For example, sartorius and semitendinosus muscles showed motor endplates that supply parallel bundles of short fibres, distributed along the muscle's belly, resulting in a scattering of the IZ throughout the muscle (Coërs and Woolf, 1959). Again, the gracilis muscle demonstrated two distinct transverse IZs (Christensen, 1959). The IZ' morphology and its exact location within muscles in the general population, have not been studied systematically, and only limited experimental data are available. Moreover, non-invasive techniques to detect the IZ, were not available until a few years ago.

A first attempt to overcome both the limitations has been made by Masuda in 1983; he started successfully to investigate the IZ of the biceps brachii muscle during a voluntary muscular contraction, using myoelectric signals (Masuda et al., 1983a). Specifically, a multichannel EMG amplifier was used and signals were picked up with an electrode array consisting of several electrodes arranged along the muscle fibre direction. (Masuda et al., 1983b). Afterwards, a number of studies applied surface electrode arrays to locate the IZ within specific muscles (Masuda et al., 1985, Iwasaki et al., 1990, Saitou et al., 2000, Falla et al., 2002). A group of Japanese researchers led a study in 17 muscles and 8 muscle groups in both the upper and lower limbs. Unfortunately, the distribution of the IZ was reported only in 3 volunteers (Saitou et al., 2000). The IZ was easily identified in all muscles having fibres running parallel to the skin for example, biceps brachii, intrinsic hand muscles, vastus lateralis and medialis, tensor fasciae latae, peronei, soleus, tibialis anterior. While in muscle with a complicated structure including pennation fibres, in-series fibres, and aponeurotic digitations, identification was difficult. The muscles involved were: deltoid, flexors and extensors in the forearm, rectus femoris, sartorius, hamstrings and gastrocnemius. Also, Shiraishi pointed to a few limitations in the localization of the IZ in muscles with complex fibre' orientations (Shiraishi et al., 1995). More recently, Rainoldi described the IZ' location in lower limb muscles and provided a method to standardise the electrode placement for those muscles (Rainoldi et al., 2004). Additionally, the IZ' shift due to different isometric contraction intensities and joint angle positions have been characterized and described in the literature (Defreitas et al., 2008, DeFreitas et al., 2010, Martin and MacIsaac, 2006, Piitulainen et al.,2009). Beck and his colleagues extensively studied the IZ' influence on sEMG variables (Beck et al., 2008a, Beck et al., 2008b, Beck et al., 2008c, Beck et al., 2007a, Beck et al., 2007b, Malek et al., 2006).

The IZ' location can be identified from sEMG signals detected using linear arrays of electrodes placed along the direction of the muscle fibres (Merletti

et al., 2003). In such configurations, the IZ appears as the area from where the potential propagates in two opposite directions towards the tendon regions (Masuda et al., 1983b). Therefore, it has been proposed that the IZ' position could be established by analysis of single differential sEMG signals based on two criteria: minimum signal amplitude and/or phase reversal. These criteria can be applied only to the single differential surface EMG signals, and not to monopolar and double differential signals, which do not show phase inversion (Merletti et al., 1999, Merletti et al., 2003).

The validity of the proposed EMG technique to identify the IZ' location, has been proposed by Masuda and Sadoyama in 1988. In their publication in Transactions on Biomedical Engineering, they illustrated how a column of electrodes receive action potentials from many muscles' fibres, and how the action potentials from a single fibre are received by several electrode columns located nearby to the fibre (Masuda and Sadoyama, 1988). The distribution of the action potential' sources was used to represent the configuration of the IZ. Notably, it was identified that surface potentials are mainly caused by muscle fibres near the skin surface, and that these fibres are only a portion of the fibres activated in the motor unit, especially in muscles with a large thickness. Additionally, although the distribution of muscle fibres is usually uniform, a variability in the distribution of fibres near the skin surface, provoke scatter of a IZ' location. These elements should be considered when applying surface EMG to describe the IZ' location. On account of the recent technological advancements, it is now possible to cover large areas of the muscle using arrays of electrodes, or even using a matrix of electrodes (Merletti et al., 2003). Thus, it is currently possible to obtain a wide, bi-dimensional measurement of the IZ' location for most skeletal muscles. To ensure an accurate application of the described technique and therefore, a valid measurement of the IZ' location, it is critical to respect two experimental elements. Firstly, it is important to select muscles with fibres parallel to the skin surface. This is because in order, to identify the IZ' location, the motor unit' action potentials should travel parallel to the columns

of electrodes. Secondly, motor unit action potentials should be detected during isometric contractions. Indeed, the IZ' location (i.e. the source of the motor unit action potentials) during a dynamic contraction, shifts under the skin according to the anatomy of the muscle under investigation, and according to the range of motion (Piitulainen et al., 2009). A shift of the motor unit action potentials' source may introduce a bias in the estimation of the IZ' location.

Being able to reliably identify the IZ within muscles has several possible applications, ranging from the simple anatomical descriptions to the more complex interventions' optimisation. Indeed, IZ localisation has been proposed in order to provide indications for botulinum toxin injection, motor point biopsy, and muscle incision during surgery (Enck et al., 2004, Merletti et al., 2003, Mesin et al., 2009, Saitou et al., 2000).

Methodologically, an analysis of how reliably the IZ can be located, is therefore, a prerequisite for further investigations concerning muscles. Two distinct issues should be addressed: the agreement between ratings made by the same observer or between different observers, and the IZ' location constancy' according to variables related to neuromuscular activation. Whenever humans are involved in a measurement procedure, it is critical to ensure that the results are reliable (Rankin and Stokes, 1998).

Reliability is related to the amount of random error in a measurement. The more reliable the measure, the less the random error in it. Estimation of the intra-observer variability is relevant when a researcher is interested in the 'true' differences among the observations made by the same observer, on the same subject. Estimation of the inter-observer variability is relevant when a researcher is interested in the 'true' differences among observers reporting different values of the same entity (Munro, 2005). Observers can be distracted, can get tired, can apply the measurement' technique in different ways, or can misinterpret the measurement' technique. This may be the case

in relation to the procedure to identify the IZ' location using EMG signals where operators are requested to conduct a visual inspection of several EMG signals, and apply two criteria (i.e. minimum signal amplitude and/or phase reversal). Moreover, during sustained isometric contractions, a few variables such as time, intensity and repetitions, may affect the pool of motor units recruited. This study conducted such an analysis within the context of a project, to determine the relationship between the IZ and myofascial trigger points in the upper trapezius muscle.

The aims of this work were to evaluate:

- The intra- and inter-rater reliability of two operators in locating the IZ in the upper trapezius muscle, using a sEMG matrix.
- The constancy of the IZ' location in relation to the contraction intensity, the acquisition period analysed, and the contraction repetition.
- Finally, the precision of matrix' repositioning by two operators, has been described.

3.3 MATERIALS AND METHODS

All the experimental sessions were conducted between October and November 2007, at the Laboratory of Engineering of Neuromuscular System and Motor Rehabilitation of Politecnico of Torino (LiSIN), Italy. The ethical approval for this study was granted by the Research Ethics Committee of Queen Margaret University (Edinburgh), and the local Regional Ethical Committee (Regione Piemonte, Italy). All experiments were conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding of the subjects. Potential participants were informed fully about the goals, procedures and risks of the study before it commenced, using an information sheet. All subjects signed an informed consent form prior to participating in any experimental procedures.

3.3.1 Participants

A convenience sample of healthy adult volunteers was invited to participate in the study using poster placed at strategic locations within LiSIN.

The following inclusion criteria were adopted to determine whether a person could participate in the study:

- Adult aged between 18 and 40 years' old
- Sex: male or female
- Body mass index below 25 Kg/m^2
- Free of neck and shoulder pain

Further, the following exclusion criteria were applied:

- Recent surgery of the upper quadrant
- Pregnancy
- Neck pain during the last 3 weeks
- Positive history for neurological disorders
- Positive history for rheumatic disorders
- Positive history for psychiatric disorders

Considering the aims of the study, it was important to enrol subjects who were able to fully activate their upper trapezius muscle during the shoulder

elevation task. Thus to avoid bias related to impaired activation of the upper trapezius muscle, only subjects who didn't report pain in the neck and shoulder prior to the experiment, were included in it. For the same reason, subjects were excluded if they suffered from any disorders that may limit their capacity to perform a shoulder elevation task.

For ethical reasons, the reported inclusion/exclusion criteria were necessary to protect subjects from physical or psychological harm. Individuals with pain or recent surgery in the neck and shoulder region, were excluded as in these subjects, upper trapezius' contractions are usually provocative. Moreover, although rare, a submaximal muscle contraction may induce pain or injuries even in healthy subjects, and especially during unusual tasks, such as repeated and intense bilateral shoulder elevation tasks. To reduce this risk, it was decided to limit the age of enrolment to 40 years, as aging may be associated with significant reduction of neuromuscular performance or function (Gouveia et al., 2013, Charlier et al., 2015, Milanovic et al., 2013). Exclusion of subjects on account of a positive history for neurological or rheumatic disorders was necessary not only to protect them from physical harm but also to exclude confounding factors related to possible neuromuscular impairments. Exclusion on account of a positive history of psychiatric for psychiatric disorders was necessary to avoid adverse psychological reactions to the experimental procedures, and also because participants may show may show altered cognitive capacity that potentially could impact upon their ability to provide informed consent. A body mass index below 25 Kg/m^2 (i.e. BMI higher the 25 generally indicates overweight or obesity) was utilised, as amplitude and the quality of myoelectric signals are significantly affected by the extent of subcutaneous fat (Hermens et al., 2000).

3.3.2 Equipment

Surface EMG signals were detected using a matrix of 64 electrodes, with 8 mm interelectrode distance (IED) and with electrodes arranged in five columns and 13 rows (one column of 12 electrodes and four of 13 electrodes, model ELSCH064, designed by LISiN at Politecnico di Torino and manufactured by OT Bioelectronica, Torino, Italy) (figure 3.3).

The electrode matrix was held in place on the skin using a piece of 1 mm thick, double adhesive foam, which contained cavities for the insertion of electrode gel. These cavities corresponded with the electrode' matrix. Each electrode cavity was injected with 20 µl of conductive gel, using a gel dispenser (Eppendorf AG-Multipette plus, Germany) to ensure proper electrode–skin contact (figure 3.3).

Surface EMG signals were amplified within a bandwidth of 10 – 750 Hz and with a gain of 1000 or 2000, depending on the surface EMG amplitude during the isometric contraction. Signals were sampled at 2048 Hz and converted using a 12 bits A/D (analog-to-digital) converter. Samples were visualized during acquisition and then stored on a personal computer using customized software developed at LISiN (Acquisition V.1.62). Force generated by the upper trapezius muscle was measured during a shoulder elevation task, with subjects in sitting position. A metallic framework with two handles attached to bars set on rails, had been fixed under a chair' seat to permit the correct subject's positioning in order to perform the task (figure 3.5). The handle on the right side had been fixed to a load cell (Mod. UU-K100, CAP 100 Kgf).
This special chair had been developed originally at the LISiN for a previous study on the trapezius muscle (Cescon et al., 2008), with a few minimal modifications of the handles needing to be done for this study.

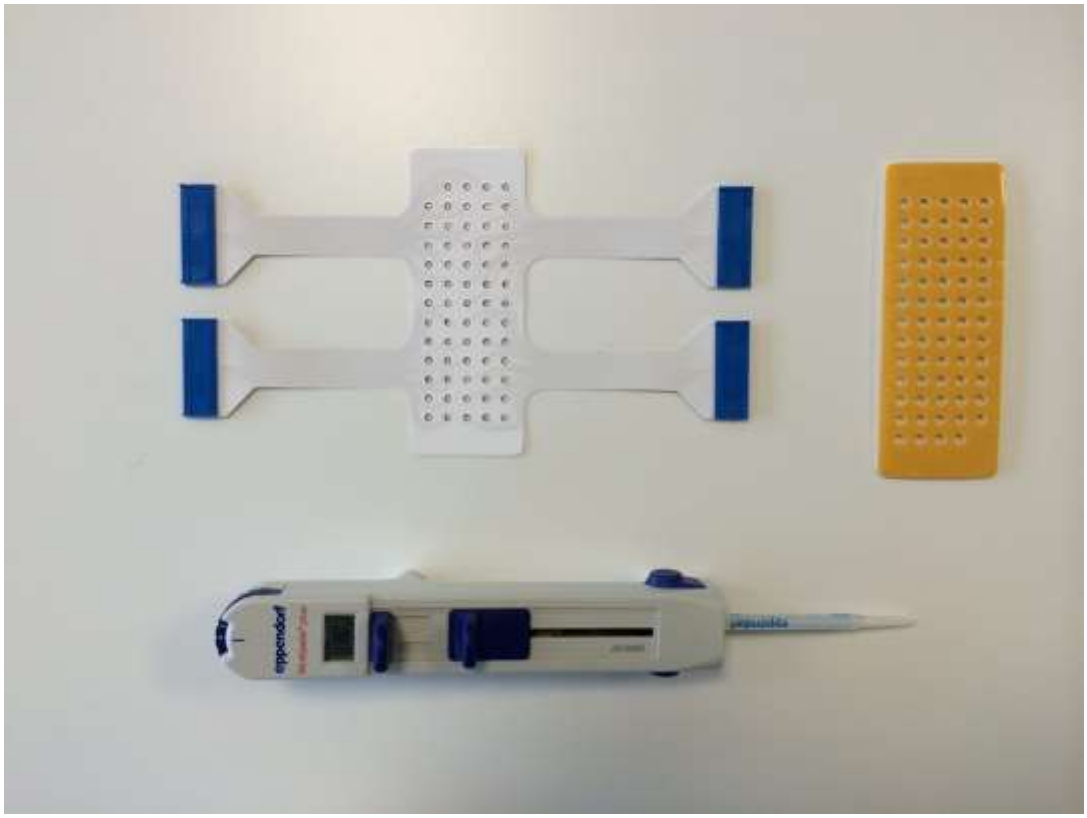

Figure 3.3: Matrix electrodes and gel dispenser. Five columns x 13 rows, one column of 12 electrodes and four of 13 electrodes, 8 mm inter-electrode distance, model ELSCH064, designed by LISiN at Politecnico di Torino and manufactured by OT Bioelectronica, Torino, Italy. On the left side is the double adhesive foam with cavities. Below, is the gel dispenser produced by Eppendorf, Germany.

Figure 3.4: EMG-USB 128 channels surface EMG amplifier. EMG-USB was designed by LISiN at Politecnico di Torino and manufactured by OT Bioelectronica, Torino, Italy.

Figure 3.5. The modified chair with the load cell.

The force signals was amplified (MISO II, LISiN, Torino, Italy, bandwidth 0-80 HZ) and visualized using a bar of 50 green LEDs (100% MVC). An orange LED was used to pinpoint the target percentage of the MVC.

In order to establish their maximum voluntary contraction (MVC) of upper trapezius muscle, subjects were asked to sit on a custom-designed chair and to hold the two chair' handles. The handle on the right side was fixed to a load cell in order to measure the force exerted during shoulder elevation. Force signals were acquired and amplified (bandwidth 0 – 80 Hz) using a MISO II amplifier (LISiN, Torino, Italy). Feedback to subjects was provided by a bar of LEDs indicating the percentage of the MVC reached during each contraction (figure 3.6).

Figure 3.6 Experimental setup. The custom made chair with a load cell connected to the right handle and a subject, with a matrix placed on the

upper trapezius, looking at the visual feedback device (MISO II, LISiN, Torino, Italy).

3.3.3 Experimental design

All the volunteers were asked to complete a case form that included the following information: age, gender, weight, height, presence of pain in the neck and shoulder region, recent surgery of the upper quadrant, pregnancy, diagnosis for neurological disorders, diagnosis for rheumatic disorders, and diagnosis for psychiatric disorders. Using the reported data, the researcher computed the body mass index (BMI) using the formula: weight in kilograms divided by the square of the height in meters (Mei et al., 2002). All of the information was self-reported; no measurements or tests were conducted to verify the anthropometric details, or the reported diagnosis.

Surface EMG signals were collected from the right upper trapezius muscle of all subjects. For technical reasons, it had been decided to collect data on the right side of the body. The equipment (i.e. the modified chair with the load cell) included only one load cell. During the experimental sessions, it was technically complex and time consuming to move the load cell from one side to the other. Given that there had been a need to be considerate of the time that research participants were asked to commit to research projects, we decided to collect all the surface EMG signals from right upper trapezius muscle. This was considered acceptable because this study focused on the reliability of IZ localization using visual inspection of motor unit' action potentials' propagation. While a different IZ' location between left and right trapezius musculature, or between dominant and non-dominant upper limbs cannot be excluded, it had been outwith the scope of this investigation. Moreover, the planned clinical study was also focus on right upper trapezius muscle.

Two physiotherapists, identified as operator A and operator B, conducted the experimental sessions. They were trained to use the described

instrumentation and to analyse the recorded data. Prior to undertaking the experimental protocol, both operators undertook a two-week training period on how to apply the criteria to locate the IZ. This training consisted of instruction in the procedure and practice, with feedback on 100 screenshots of eleven single differential surface EMG signals (i.e. channel) from different skeletal muscles. Each screenshot included one EMG epoch of 0.25s and the operators had to score the IZ' location according to the available channels. Both training and feedback were provided by a senior researcher (Lorenda Lo Conte), proficient in biomedical signal processing and employed for more than 10 years at the Laboratory of Engineering of Neuromuscular System and Motor Rehabilitation of Politecnico of Torino (LiSIN). The training was completed successfully and both the operators were able to correctly apply the criteria to identify the IZ' location.

Prior to collecting surface EMG data, in order to measure the MVC, each subject sat with the trunk against the chair back, arms by the side, hands grasping the two handles, and feet hanging in the air. The subject was instructed to perform a shoulder elevation task by pulling upwards on both handles simultaneously (to ensure trunk stability) and without moving the shoulder girdle, so that the activation of the trapezius muscle was indeed due to an isometric contraction. The co-activation of the levator scapulae muscle was considered to be not relevant due to its anatomical position with respect to the upper trapezius muscle (i.e. medial and ventral). Indeed, the peak potential amplitude of motor unit' action potentials decays drastically within 20 to 25 mm from the source (Farina et al., 2002). Considering that the upper trapezius' thickness at the level T1 is about 15 mm (O'Sullivan et al., 2009), the distance of the levator scapula from the surface electrodes can be 20-30 mm approximately. Moreover, the motor unit' action potentials of the levator scapula muscle travel in a different direction with respect to the upper trapezius fibres (caudal-cranial for levator scapulae muscle and medial-lateral for upper trapezius muscle), and can only minimally affect the amplitude of the EMG from the upper trapezius muscle.

Before starting the experiment, each subject learnt how to use the force feedback tool to calibrate the force exerted, according to the level requested by the operator. The reference MVC force' level for each subject was determined as the maximum of three contractions, with each contraction being followed by 2 minutes of rest. Subjects were verbally encouraged during the 3 MVCs.

An important factor affecting the impedance of the electrode-gel-skin interface is skin. It has a large impendence to current flow due to the upper layer of the epidermis, the stratum corneum. According to a study published by Kim in 1989, a mild abrasion of the skin with fine sandpaper can reduce the skin resistance by a factor 100 to 1000 (Kim, 1989). The European Project on Surface EMG for Non Invasive Assessment of Muscles (SENIAM) recommends that the skin procedures consist of shaving, massaging with sandpaper or abrasive paste and rinsing the skin with isopropyl alcohol to remove the abrasion residuals (Hermens et al., 2000). Therefore, oils and flaky skin layers were removed from the skin of each of the participants in this study by abrasive paste (Spes Medica srl, Genoa, Italy).

For each subject, a landmark system to place the electrode matrix according to the anatomical description of the upper trapezius muscle, had been agreed on by the two operators (Hermens, 1999). Subsequently, one operator traced with a surgical pen, a first line joining the C7 vertebra and the acromial angle, and a second line perpendicular to the midway position of the first one. The so defined orthogonal system was used to place the center of the matrix on the intersection point of the two lines (figure 3.7).

Following this, sEMG acquisition started. Each subject performed six contractions that was overseen by operator A and six contractions with operator B. The operator's order was randomized. Randomization was performed using sealed opaque envelopes: 10 for the subjects, 2 for the

operators and 6 for the contraction's order. Each contraction lasted 10 s. Three of these contractions were at 20% MVC and three were at 40% MVC. Again, the order of the contractions was randomized.

After a session of six contractions, the electrode matrix was removed and repositioned by the second operator for the following session. The landmark system previously drawn on each subject was not erased between the matrix' repositioning.

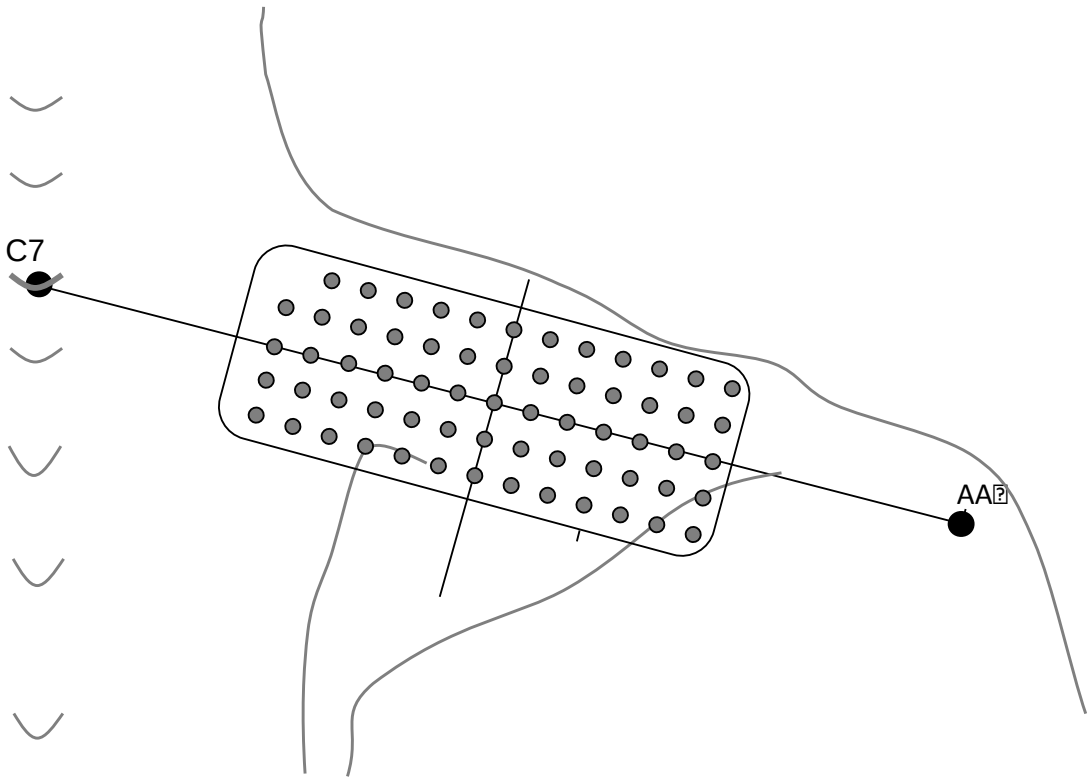

Figure 3.7: Matrix' position according to the anatomical landmark system, with columns oriented with respect to the direction of upper trapezius fibres. C7 indicates the spinous process of the seventh cervical vertebra. AA indicates the acromial angle of the scapula.

3.3.4 Data

Overall 600 signals were available for the analysis (2 operators x 10 subjects x 6 contractions x 5 columns). Signals were acquired in single differential mode.

In order to address the main questions of this work, we randomly extracted 900 epochs of 0.25s of sEMG signals from each of the matrix columns. Epochs of 11 single differential surface EMG signals were presented to the two operators for visual analysis. Each file reported signals from 11 numbered channels. Each signal corresponded to the differences between the signals collected by two consecutive electrodes within the same column (figure 3.8 and 3.9). Using customized software, we created five epoch lists:

List number 1 (L_Reliability) was used to assess the intra-rater and the inter-rater reliability in locating the IZ' position. One hundred epochs collected by operator A and 100 by operator B, including 100 at 20% MVC and 100 at 40% MVC, were extracted. All the epochs were from 5.00s to 5.25s. The latter epochs were randomly extracted from among the 600 acquisitions (appendix I).

List number 2 (L_Intensity) was used to assess if the intensity of the isometric contraction had an influence on the IZ' location. One hundred epochs collected by operator A were paired together, and these included 50 epochs at 20% MVC and 50 epochs at 40% MVC. These epochs were randomly extracted from the 600 acquisitions obtained from all the enrolled subjects, from 5.00s to 5.25s (appendix II).

List number 3 (L_Time) was used to assess if the IZ' location was stable over 10s of isometric contraction. Two hundred epochs collected by the two operators were extracted randomly. Fifty signals were randomly selected and for each of the signals, four epochs were randomly extracted (appendix III).

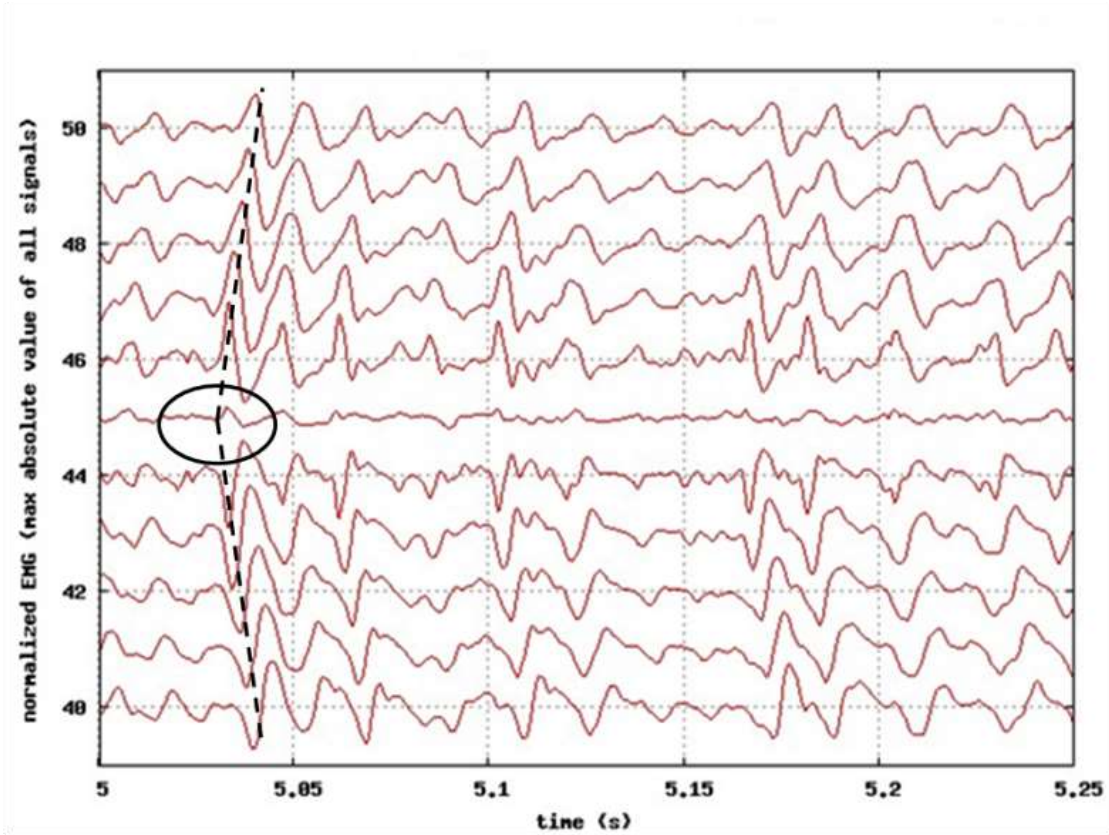

Figure 3.8: Eleven SD sEMG signals over a 0.25 s epoch, collected from channel 40 to channel 50 of the array. Dotted lines represent motor unit action potential' propagation. The circle indicates the IZ' location estimated by the operator (channel 45) and scored as 45.

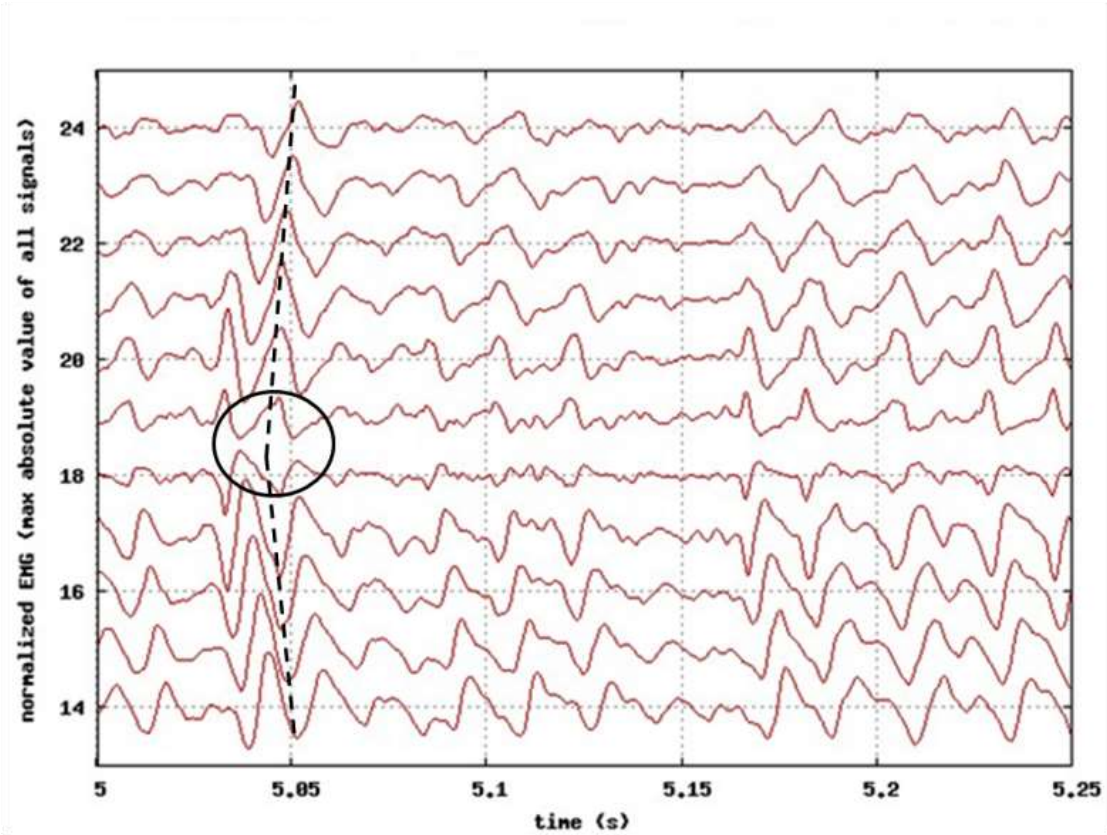

Figure 3.9. Eleven SD sEMG signals over a 0.25 s, collected from channel 14 to channel 24 of the array. Dotted lines represent motor unit action potential' propagation. The circle indicates the IZ' location identified by the operator between channel 18 and channel 19, and scored as 18.

List number 4 (L_Repetition) was used to assess whether repetitive contractions had an effect on the IZ' location. Three hundred epochs collected by operator B were extracted. Signals from the same columns in the 10 subjects, during the six contractions, were considered. All the epochs were extracted from 5.00 s to 5.25 s (appendix IV).

Finally, list number 5 (L_Repositioning) was used to assess the precision of matrix' repositioning by the two operators, using the same anatomical landmark system (ALS). One hundred epochs collected by operators A and B were extracted randomly. All of the first 20% MVC signals from the same column were considered (2 operators x 10 subjects x 5 columns) (appendix V and VI).

Criteria applied by the customized software to extract the epochs from the five lists, are summarized in the table 3.1. All the above epoch's lists were provided to the two operators for the visual analysis. Each operator repeated the procedures three weeks later.

3.3.5 Localization of the innervation zone

The two operators estimated the IZ' location by visual analysis, independently from each other. Each selected epoch (a set of 11 SD signals) was visually analysed to identify the IZ' area, as the one corresponding to the signal with minimum amplitude and/or phase reversal (Merletti et al., 1999). If the minimal amplitude channel was between two channels showing phase reversal (figure 3.8), the IZ was located in correspondence of that channel. If two channels showing phase reversal were adjacent (figure 3.9), the IZ was located as the middle area between these two channels. In this case, the operator actually had to interpolate between the two signals.

Since the interelectrode distance was 8 mm, and given the interpolation, the resolution with which each operator estimated the IZ' location was 4 mm, or

half the interelectrode distance. Possible scores for the IZ' locations for each column, are reported in figure 3.10.

Table 3.1: Criteria to extract the epochs for the five lists.

List	Aim	EMG Acquisitions	Operator (A or B)	MVC (20% or 40%)	Time (0 to 10 s)	Repetitions (1 to 6)
Number 1	Intra and inter rater reliability	40 EMG acq. RND selected (200 epochs)	20 acq. Form A 20 acq. From B	10 acq. at 20% and 10 acq. at 40%from A 10 acq. at 20% and 10 acq. at 40%from B	Fixed	RND
Number 2	Effect of isometric contraction intensity	1 EMG acq. For each subject (100 epochs)	A fixed	50 epochs at 20% and 50 epochs at 40% from the same session	Fixed	RND
Number 3	Effect of epoch;s time extraction	10 EMG acq. RND selected (200 epochs)	RND	RND	4 RND epochs for each column of the selected acq.	RND
Number 4	Effect of contraction repetition	All EMG acq. for each subject (300 epochs)	B fixed	20% and 40%	Fixed	All the 6 contractions for each subjects
Number 5	Effect of matrix repositioning	1 EMG acq for each session (100 epochs)	A and B	20%	Fixed	Fixed, first 20% repetition

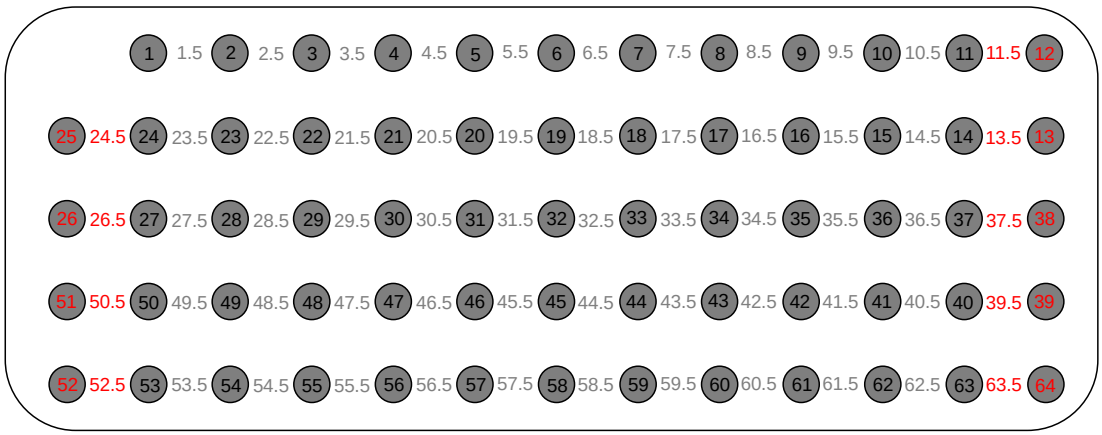

Figure 3.10: Score system for the IZ' location according to the electrode' matrix. Red numbers, which were not available as surface EMG signals, were detected in single differential mode.

3.3.6 Statistical analysis

The agreement among estimates in each list, was reported by absolute values and percentage. Intra-rater and inter-rater reliability (L_Reliability) were assessed using Cohen's Kappa statistics (Cohen, 1960), as the IZ' location, measured using surface EMG signals (detected with the linear array) should be considered a nominal variable. Indeed, IZ' location has been described considering two possible positions among the 11 channels: corresponding to the position of one channel or between two consecutive channels.

The Kappa coefficient is more robust than the percent agreement calculation, since it takes into account the agreement occurring by chance. The constancy in IZ' localization has been quantified as the number and entity of the disagreement regarding the IZ' position, when considering variables related to neuromuscular activation.

In order to run the Cohen's Kappa statistics, the following assumptions were met: judgement made by the two raters must be measured on an ordinal or nominal scale; judgement data must be paired observations of the same phenomenon; each judgement variable must have the same number of categories; the two raters must be independent and fixed (Cohen, 1960).

Prior to undertaking the study, sample size was obtained using a table based on a goodness-of-fit formula provided by Donner and Eliasziw (1992). Considering our 2-rater study, the minimum number of IZ' estimates (i.e. epochs) required to detect a kappa coefficient as statistically significant ($p < 0.05$), with 90% power, was 30. It was assumed a null hypothesis value of Kappa equal to .00 and a Kappa to detect, of 0.60 (appendix VII).

3.4 RESULTS

Ten volunteer subjects, seven males and three females, all right-handed, participated in this study. Subjects' characteristics are summarized in table 3.2. The operator's order and contraction's order was randomized prior to the data collection (appendix VIII). Absolute frequency of IZ is reported for each possible location on the electrode' matrix, is summarized in figure 3.11.

Intra-rater reliability analysis (L_Reliability) indicated an ''almost perfect agreement'' (Interpretation of the Kappa coefficient: 0.01 – 0.20 slight agreement, 0.21 – 0.40 fair agreement, 0.41 – 0.60 moderate agreement, 0.61 – 0.80 substantial agreement, 0.81 – 0.99 almost perfect agreement, for both operators) (Cohen, 1960); the percent agreement for operator A was 91.5% (table 3.3), with Kappa = 0.90, and the percent agreement for operator B was 93% (table 3.4) with Kappa = 0.92. Inter-rater reliability analysis indicated an 'almost perfect agreement'' between the two operators. The percent agreement was 85% (table 3.5) of estimates, with Kappa = 0.82.

The variations on IZ' localization for the remaining lists were as follows: no variation in 76% (38 out of 50 epochs) of the estimates in L_Intensity, no variation in 66% (33 out of 50) of the estimates in L_Time, no variation in 50% (25 out 50 epochs) of the estimates in L_Repetition, and no variation in 34% (17 out 50 epochs) of estimates in L_Repositioning. The results are summarized in table 3.6 and table 3.7.

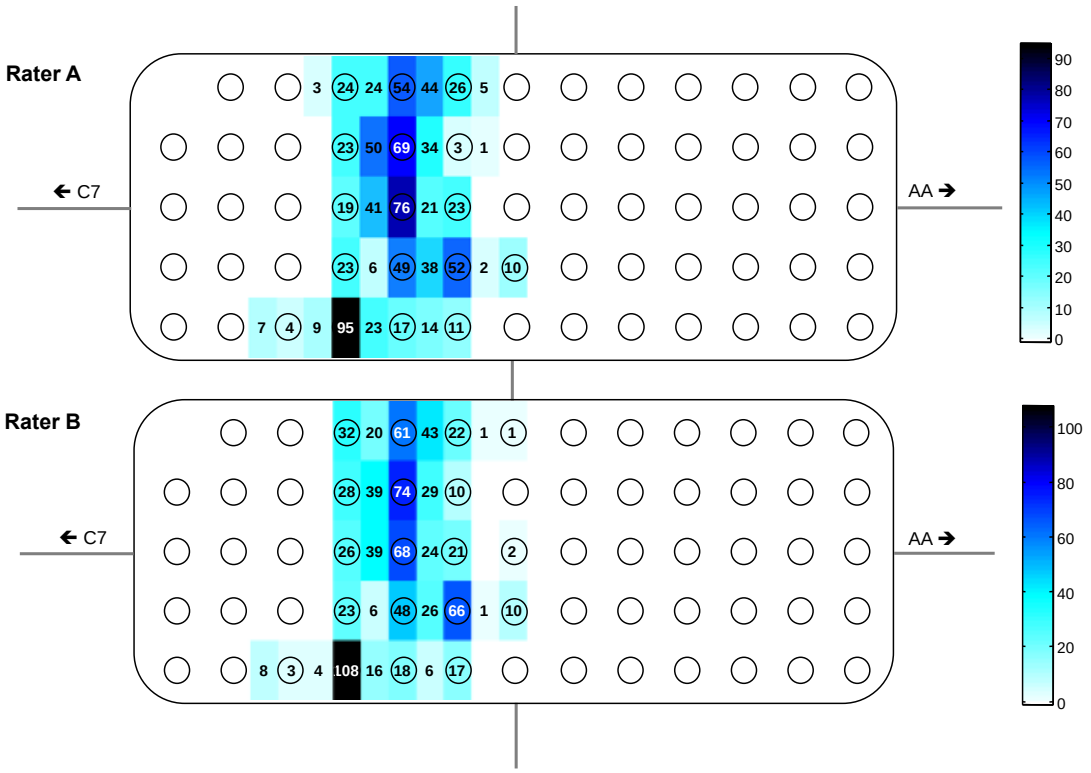

Figure 3.11: IZ' locations estimated by the two raters using the proposed score system. Absolute frequency of IZ is reported for each possible location on the electrode' matrix. Both the operators examined the same 900 epochs of 0.25 s.

Table 3.2: Subjects' characteristics. Values are expressed as mean ± SD except for gender. F: female, M: male, BMI: body mass index, C7 to AA: distance between C7 vertebra and the acromial angle, MVC: maximal voluntary contraction.

Subject_ID	Age (years)	Gender	Height (m)	Weight (Kg)	BMI (Kg/m^2)	C7 to AA (cm)	MVC (N)
1	37	F	1.63	55	20.7	18.5	412
2	24	M	1.82	82	24.7	24	666
3	23	M	1.85	73	21.3	24	735
4	27	M	1.73	74	24.7	22	637
5	27	M	1.78	78	24.6	23.5	676
6	33	M	1.86	82	23.7	21.5	676
7	21	M	1.75	69	22.5	20	490
8	25	F	1.58	50	20	20	382
9	27	M	1.75	68	22.2	22.7	666
10	40	F	1.68	52	18.4	19	529
	28.4 ± 6.2	3F, 7M	1.7 ± 0.1	68.3 ± 12	22.3 ± 2.2	21.5 ± 2	586 ± 124

Table 3.3: Intra-rater percentage agreement for operator A during IZ' estimations**.**

Column	Epochs	Agreement	Disagreement
1	40	35	5
2	40	37	3
3	40	36	4
4	40	38	2
5	40	37	3
Total	200	183 (91.5%)	17 (8.5%)

Table 3.4: Intra-rater percentage agreement for operator B during IZ' estimations.

Column	Epochs	Agreement	Disagreement
1	40	39	1
2	40	37	3
3	40	37	3
4	40	36	4
5	40	37	3
Total	200	186 (93%)	14 (7%)

Table 3.5: Inter-rater percentage of agreement for operator A and B during IZ' estimations**.**

Column	Epochs	Agreement	Disagreement
1	40	32	8
2	40	33	7
3	40	34	6
4	40	35	5
5	40	36	4
Tot	200	170 (85%)	30 (15%)

Table 3.6: Summary of the results, reliability analysis. IED: inter-electrode distance, IZ: innervation zone. (1 and 2):Intra-rater agreement and disagreement (number of cases and percentage) and K values, (3) inter-rater agreement and disagreement (number of cases and percentage) and K values.

Row	List's name	Estimates		K statistics
		Agreement	Disagreement	K values
1)	L_Reliability, rater A (Epochs considered: 200, two estimates for each epoch)	186 (93%)	14 (7%)	0.90
2)	L_Reliability, rater B (Epochs considered: 200, two estimates for each epoch)	170 (85%)	30 (15%)	0.92
3)	L_Reliability rater A vs B (Epochs considered: 200, two estimates for each epoch)	38 (76%)	12 (24%)	0.82
4)	L_Intensity (Epochs considered: 100, 50 at each level, one rater)	33 (66%)*	17 (34%)**	-
5)	L_Time (Epochs included: 200, 50 groups of 4 epochs, one rater)	25 (50%)*	25 (50%)**	-
6)	L_Repetition (Epochs included: 300, 50 groups of 6 epochs,one rater)	17 (34%)	33 (66%)	-
7)	L_Repositioning (Epochs included: 100, 50 for each of the two applications, one rater)	183 (91.5%)	17 (8.5%)	-

* No IZ' shift observed within the epoch's group . ** At least one IZ' shift observed within the epoch's group

Table 3.7: Summary of the results, disagreement and IZ' shift analysis. IED: inter-electrode distance, IZ: innervation zone.

Row	List's name	Extent of disagreement or observed IZ' shift (number of cases)			
		0.5 IED	1 IED	1.5 IED	2 IED
1)	L_Reliability, rater A (Epochs considered: 200, two estimates for each epoch)	15	2	0	0
2)	L_Reliability, rater B (Epochs considered: 200, two estimates for each epoch)	13	0	0	1
3)	L_Reliability rater A vs B (Epochs considered: 200, two estimates for each epoch)	28	1	0	1
4)	L_Intensity (Epochs considered: 100, 50 at each level, one rater)	10	2	0	0
5)	L_Time (Epochs included: 200, 50 groups of 4 epochs, one rater)	16	1	0	0
6)	L_Repetition (Epochs included: 300, 50 groups of 6 epochs,one rater)	20	4	0	1
7)	L_Repositioning (Epochs included: 100, 50 for each of the two applications, one rater)	20	12	1	0

Additional notes for table 3.7 and 3.8.
(1 and 2) Intra-rater agreement and disagreement (number of cases and percentage), entity of disagreement expressed in number of cases showing disagreement of 0.5, 1, 1.5, and 2 IED, Kappa value (Cohen 1960).
(3) Inter-rater agreement and disagreement, as above.
(4) Agreement and disagreement between two estimates of the IZ' location (by one rater) at two contraction intensities (20% MVC and 40% MVC).
(5) Agreement and disagreement among four estimates of the IZ' location (by one rater) using four epochs from the same signal.
(6) Agreement and disagreement among six estimates of the IZ' location (by one rater) using six epochs from six consecutive contractions.
(7) Agreement and disagreement between two estimates of the IZ' location (by one rater, for 10 subjects and for each column of the matrix) after two matrix applications on each subject (10 subjects x 5 columns x 2 conditions).

3.5 DISCUSSION

Recently, a few methods have been proposed to automatically estimate the IZ' location (Beck et al., 2012, Enck et al., 2010, Mesin et al., 2009, Ostlund et al., 2007). A test set of sEMG signals for which the IZ has been located by expert raters, can be used as the ''gold standard'' against which to test automated methods.

During this study, the raters successfully scored 900 epochs, providing IZ' location for examined epochs. Prior to this IZ' evaluation, the raters completed a specific training. The results regarding intra- and inter-rater reliability showed that two trained operators exhibit a high rate of agreement in locating the IZ, using the information provided by single differential sEMG signals acquired with an electrode matrix. All the Kappa values were in the ''almost perfect agreement'' range. There is only a slight difference between intra- and inter-rater reliability and, as expected, the chance of agreement is greater in the intra-rater reliability' case.

Considering the degree of disagreement, the intra-rater results show that, with the exception of three estimates out of 31 (two by operator A, one by operator B), the operators disagree by half a channel (15 estimates for operator A, 13 for operator B). Similarly, in 28 cases out of the 30 for which the two operators disagree (inter-rater disagreement), they do so by half a channel (table 3.7). Four millimeters (half IED) is the minimum disagreement that can be detected, given the experimental conditions.

This half-channel disagreement points to the operator's difficulty in discriminating between an IZ under a single channel or between two consecutive channels. A review using visual assessment showed that these half channel disagreements were usually present in epochs having more than one channel with reduced amplitude and with a phase that hadn't been discerned easily. An example of disagreement between raters is reported in figure 3.12. This condition makes the identification of a clear phase reversal

somewhat more difficult. This phenomenon could be due either to positions in which there is a high electrode–skin impedance, or to an IZ that had been wider than the IED.

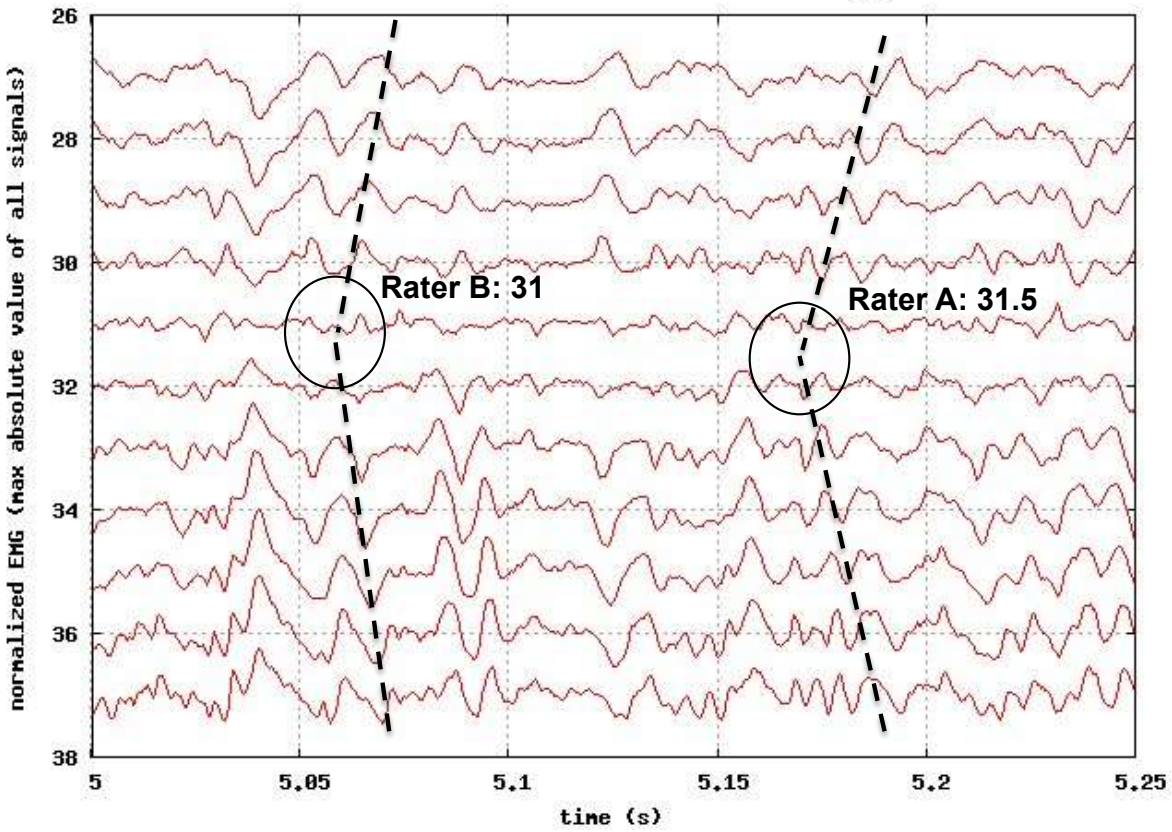

Figure 3.12: Example of disagreement of half an IED between the raters.

Once more, the results highlight the importance of proper skin treatment to reduce as much as possible, skin impedance prior to any sEMG' acquisition (Merletti et al., 2009).

The remaining lists (L_intensity, L_time, L_repetition, L_repositioning) clearly indicate an increased discordance between estimates with respect to intra-rater and inter-rater reliability (L_reliability), in the range of 34 – 66% (table 3.6). This suggests that variables affecting populations of active MUs (or related to the signal acquisition' procedures) could affect the IZ' location constancy', determining a shift of the estimated location.

The comparison between 20% MVC and 40% MVC epochs (L_intensity list) showed that force intensity could affect the IZ' location, possibly because the newly recruited motor units have different IZs. The difference between estimates (table 3.7) is one channel in two cases, and in 10 cases half a channel. A minimal IZ' shift due to the force levels and joint angle, has been already reported for the vastus lateralis, vastus medials, and the biceps brachii muscle (Martin and MacIsaac, 2006, Rainoldi et al., 2000), where the resolution of the IZ shift is determined by the IED of the used array. For example, the biceps brachii IZ' shift, using a 15-channel array with 2.5 mm IED, has been quantified as varying between 4.5 and 7 mm with increases in isometric force and independently, with increasing joint angle (Defreitas et al., 2008). Our results show that the same phenomenon could also be observed in the upper trapezius, and be explained either by the shortening of the muscle fibres and lengthening of the tendons (Piitulainen et al., 2009), or by the recruiting of MUs with slightly different IZ' locations (Gazzoni et al., 2001).

The level of disagreement (in percentage) for the L_time and L_repetition (row five and six of table 1) is higher than that reported in row one to four. This higher value accounts for the intra-rater reliability (rows one and two of table 1) that affects each of the comparisons between pairs of the four (six

pairs according to the combinatorics) or six epochs (15 pairs according to the combinatorics). Indeed, the expected intra-rater agreement using four or six epochs, should be lower. Considering four estimates (always the same epoch), the expected percentages of agreement for operator B will be 0.93 (93%) to the power of six, which correspond to 0.64 (64%). The same calculations for six epochs result in 0.34 (34%).

Therefore, it is not possible to simply conclude that the durations of the isometric contractions and their repetition, have an effect on the IZ' location constancy, as these changes could well be due, at least in part, to human uncertainty in determining the IZ.

The last list (L_positioning) shows a relatively high discordance level in IZ' location after electrode matrix' repositioning, with a difference between estimates of one or more IED in 13 out of 50 IZ' location' estimates (table 3.11). This indicates that the matrix' placement has been a difficult step of the experimental procedure, even though the landmark system was the same. Positioning should be performed with extreme attention, carefully centering and aligning the matrix with respect to the landmark system that had been drawn on the subject's skin. This could be difficult in some subjects, as the upper trapezius area is not always a flat surface and the electrode matrix is only partially flexible. However, it should be noted that the positioning of the matrix does not influence the reliability of an operator in estimating the IZ' location.

3.6 LIMITATIONS OF THE STUDY

As mentioned previously, the validity of surface EMG in locating the IZ was originally described in the eighties (Masuda and Sadoyama, 1988). Nevertheless, its validity can be limited by two factors: the ability to adequately cover the muscle belly and the execution of isometric contraction. The experimental procedures attempted to control both of these factors. The

electrode matrix that was used was the largest available on the market, covering an area of 30.72 cm2 (9.6 cm x 3.2 cm). Given the anatomy of upper trapezius muscle, this should be sufficient, but it is acknowledged that a complete covering of trapezius muscle cannot be guaranteed and hence, a partial localization of the IZ cannot be excluded. Whilst consideration was given to ensuring that the IZ' location was established during a shoulder' elevation task that used a standardised positioning of subjects, it is recognised that small changes of posture may have occurred during the contractions, which may have induced an IZ' shift during the EMG' recording. However, an investigator carefully monitored the subjects' posture to ensure a consistent starting posture and performance during the task.

In this reliability study, the Kappa statistic had been used as the IZ' position was considered to be a nominal scale with two possible values (i.e. on one channel, or between two channels) among the 11 channels. Additionally, the frequency, the size and the range of the disagreement were explored using descriptive statistics (Sim and Wright, 2005). The epoch's sample (i.e. 200) largely exceeded the minimum required (i.e. 30) assuming a null hypothesis value of Kappa equal to .00, but epochs were randomly extracted from EMG' measurements performed on 10 subjects. Their age ranged from 21 to 40 years and their BMI from 18.4 to 24.7. A sampling error cannot be excluded; a larger number of subjects with different characteristics may lead to a different epoch's sample. The quality of EMG' signals may be affected by aging or anthropometrics characteristics of the subjects; in particular, an increase of subcutaneous fat layer can induce a reduction of the signal' amplitude an increase of the cross-talk' effect (Merletti and Parker, 2004). This may lead to a difficulty in applying the visual analysis and thus, an increase of disagreements between operators.

Signals were detected using a matrix of 64 electrodes (5 columns and 13 rows) with 8 mm IED that covered an area of 30.72 cm^2 (9.6 cm X 3.2 cm). Each matrix was carefully placed on a standardized ALS assuming that fibres

were parallel to the matrix's columns. An alternative method for the matrix' placement can potentially ensure a better alignment between muscle fibres and matrix columns. This could facilitate the representation of the motor unit' action potential' propagation and thus, potentially improve the application of the visual analysis for the IZ' detection.

The 8 mm IED limited the accuracy of the IZ' estimations to 4 mm (i.e. half IED). This level of accuracy was deemed suitable for this experiment's purpose but it could be inadequate for different applications, such as for example, surgical incisions or botulinum toxin injections. Matrices or arrays of electrodes with a lower IED (i.e. 5mm or 2.5 mm) are commercially available but generalization of results of this study to different methods in order to detect the EMG' signal, should be made with caution.

For a similar reason, the results cannot extend from the upper trapezius muscle to different muscles. The methodology can be applied to other muscles with an arrangement of parallel fibres but not to muscles with pennate fibres' arrangements, in which motor units' action potential propagation can be poorly visualized using surface EMG (Barbero et al., 2012b, Beretta Piccoli et al., 2014). The upper trapezius muscle may be considered an optimal muscle to test the proposed method, but reliability could be lower in other muscles.

3.7 CONCLUSIONS

This study provides strong evidence that the visual estimation of the IZ' location using sEMG' signals, acquired by an electrode' matrix, is a reliable procedure. This is a necessary prerequisite for a planned investigation on the spatial relationship between the IZ and myofascial trigger points in the upper trapezius muscle. This methodology could be also considered for use in other potential applications, such as determining the physiological

characteristics of muscles, optimising applications of functional electrical stimulation, and guiding botulinum toxin injection, motor point biopsy, or muscle incision during surgery. Moreover, manual annotations can be used to compare and validate the output of algorithms that determine the IZ' location automatically. These results also show that the resolution in locating the IZ of the trapezius is half IED. This conclusion may or may not be generalized to other muscles with different innervations' morphology.

CHAPTER 4

INTRA-RATER RELIABILITY OF AN EXPERINCED PHYSIOTHERAPIST IN LOCATING MYOFASCIAL TRIGGER POINTS IN UPPER TRAPEZIUS MUSCLE

4.1 SUMMARY

Myofascial trigger points (MTrPs) are considered the principal clinical feature of MPS. A MTrP consists of spot tenderness within a taut band of muscle' fibres and its stimulation can produce both local and referred pain. The clinical diagnosis of MPS depends on correct history' taking and a physical examination aimed at identifying the presence of a MTrP. The purpose of this study was to investigate the intra-rater reliability of a palpation protocol used for locating an MTrP in the upper trapezius muscle.

Twenty-four subjects (23 female and 1 male; age 24 ± 3 years) with a MTrP in the upper trapezius muscle were examined by an experienced physiotherapist. During each of eight experimental sessions, subjects were examined twice in randomized order, using a palpation' protocol. An anatomical landmark system was defined and the MTrP' location established using X and Y values.

The intraclass correlation coefficient, $ICC_{(1,1)}$,values were 0.62 (95% CI: 0.30 – 0.81) for X and 0.81 (95% CI: 0.61 – 0.91) for Y. The Bland–Altman plots for X and Y showed a mean of difference of 0.04 and 20.2 mm, respectively. Limits of agreement for X ranged from 26.3 to 26.2 mm, and for Y from 27.0 to 26.4 mm.

The $ICC_{(1,1)}$ for the observed values revealed a moderate to high correlation, and the Bland–Altman analysis showed means of difference that were very close to zero, with narrow limits of agreement. An experienced physiotherapist can reliably identify MTrP' locations in upper trapezius muscle using a palpation protocol.

4.2 INTRODUCTION

Pain is a ubiquitous and mutable symptom for patients in all the branches of medicine. Musculoskeletal pain perceived in different regions (neck pain, shoulder pain, knee pain, low back pain, joint pain, chronic widespread pain) is a major reason for consultation in primary care (Main, 2005). Its assessment is a complex step of the patient's examination and clinicians need a solid clinical reasoning to understand its nature, characteristics, and sources. Once they have completed the history' taking, clinicians usually apply self-report questionnaires to quantify the patient's disability, to the test fro kinesiophobia, or to measure the patient's quality of life. Additionally, physical examination (i.e. orthopaedic tests, neurological tests, manual palpation) may be performed to identify specific impairments or to diagnose medical conditions. Despite the recent technological advances in diagnostic procedures, manual examination still remains a core competency for physiotherapists and health care' practitioners. Manual palpation skills if applied correctly, provide relevant information including the bony location, tissue temperature, and texture. Again, these competences are also important for clinical reasoning and manual therapy treatments (Smart and Doody, 2007).

Physical examination is generally manually-applied and the test's positivity is mainly the painful response. It can be a pain from a specific anatomical region or the pain usually complained by the patient. Table 4.1 reports a few examples of pain provocation' tests for the upper limb, together with criteria for positivity (O'Brien et al., 1998, Lozman et al., 1995, Cordasco et al., 1993, Bhargava et al., 2010, Hegedus et al., 2008). All of the tests underwent specific investigations aimed at confirming their validity and reliability. The standard methodological approach for validity is to explore the correlation between the pain provocation and the presence of the pathological conditions (Farber et al., 2006, Ben Kibler et al., 2009). By contrast, reliability studies focus on the pain provocation consistency of the manoeuvres (Marx et al., 1999, Cadogan et al., 2011, Kelly et al., 2010). The rationale of the

provocative manoeuvres (i.e. the manual procedures) is constructed by considering the target condition, especially its anatomical location and its pathophysiology. The underling mechanism is usually a mechanical stress (i.e. compression or strain), directed to the anatomical structures affected by

Table 4.1: Orthopaedic tests. All the reported tests are considered pain provocation tests as their positivity rely on pain provocation.

Name	Name	Purpose	Positivity
O'Brien 1998	Active Compression test	Test the integrity of the glenoid labrum of the shoulder	Painful clicking "inside" the shoulder
Lozman 1995	Cross Over Test	Test lesion or dysfunction at the acromioclavicular joint	Reproduction of the patient's painful symptoms
Bhargava 2010	Cozen's Test	To assess the lateral elbow for tendinopathy	Reproduction of the patient's painful symptoms
Cordasco 1993	Anterior Apprehension Test	To diagnose the glenohumeral joint instability	Patient complains of pain or instability
Hegedus 2008	Hawkins Test	To diagnose shoulder impingement syndromes	Shoulder Pain

the pathological condition. In line with the rationale described above, the manual palpation procedures to diagnose the MPS, are aimed at evoking painful symptoms from a MTrP, which is indeed considered a pathognomonic sign of the MPS.

MTrPs have been described as discrete areas of muscle tenderness, presenting in taut bands of muscle, with a diagnosis dependent on a correct history' taking, and confirmed by a physical examination. Three minimum clinical diagnostic criteria have been proposed: indurated bundle of fibres within a muscle, known as taut band; focal hypersensitivity and a painful point in the taut band, called spot tenderness; and a referred pain sensation with mechanical stimulation of the spot tenderness, known as referred pain (Simons et al., 1999, Mense et al., 2001). An additional six confirmatory features may be present: local twitch response with snapping palpation of the taut band, jump sign, patient recognition of the elicited, predicted referred pain' patterns, muscle weakness or muscle tightness, and pain with stretching or contraction of the affected muscle (Simons, 2004, Cummings and Baldry, 2007, Bennett, 2007).

A physical examination is used to confirm a MTrP diagnosis, which consists of a palpation protocol that includes manual palpation, and the patient's replies to specific questions about elicited painful symptoms.
Numerous research studies and two systematic reviews (Lucas et al., 2009, Myburgh et al., 2008) have been conducted to investigate the reproducibility of the MTrPs' examination for several muscles (Nice et al., 1992, Njoo and Van der Does, 1994, Lew et al., 1997, Gerwin, 1997, Hsieh et al., 2000, Bron et al., 2007, Al-Shenqiti and Oldham, 2005, Wolfe et al., 1992). These studies have focused on the reliability of the MTrPs' diagnostic criteria, with no attention given to the reliability of palpation protocols in identifying the MTrPs' exact location. Indeed, all the reliability studies included in the systematic reviews assumed that the operators had applied the diagnostic criteria to the same MTrP (i.e. with the same location), but this condition was

not controlled. Overall, the best reproducibility of the MTrP diagnostic' criteria has been reported in the upper trapezius muscle, which is frequently affected by MTrPs, as observed in patients with neck pain and chronic tension-type headaches (Fernandez-de-las-Penas et al., 2007, Unalan et al., 2011, Fernandez-de-las-Penas et al., 2006a). These should be considered important elements for any investigation on the accuracy of manual palpation in locating MTrPs, and for the feasibility of the proposed project. Indeed, the final aim of this project will include an accurate localization of both the IZ and MTrP in upper trapezius muscle. The relevance of this investigation could be also extended to MTrP' treatment that requires the same MTrP to be located and treated over repeated sessions. The aim of this study was to investigate the intra-rater reliability of a palpation protocol, performed by an experienced physiotherapist, in locating a MTrP in the upper trapezius muscle.

4.3 MATERIALS AND METHODS

Experimental sessions were conducted between November and December 2011, in the laboratory of movement analysis at Vita-Salute San Raffaele University, Milan, Italy. The ethical approval was granted by the Research Ethics Committee of Queen Margaret University (Edinburgh) and, by the Internal Ethics Committee of the San Raffaele Hospital of Milan (Italy). All experiments were conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding of the subjects. The study respected the Ethical Guidelines for Pain Research in Humans (Charlton, 2005). Potential participants were informed fully about the goals, procedures and risks of the study, before the study, using an information sheet. All subjects, prior to participating in any experimental procedures, signed an informed consent form.

Two junior physiotherapists and one senior physiotherapist, participated in the study. The experienced physiotherapist had 10 years of clinical

experience and a specialism in the management of MPS. Additionally, he had attended postgraduate courses on MTrP' diagnosis and treatment. Before the study, the junior physiotherapists completed a training period in MTrP' palpation under the supervision of the experienced physiotherapist. Specifically, the training focused on the flat palpation technique for upper trapezius muscle, and on the ability to identify both the taut band and spot tenderness. Pincer palpation is also possible for locating the MTrP in the upper trapezius muscle, but considering the experimental setup of this study (i.e. blinding of the operator and sitting position), the operator was asked to apply only the flat palpation technique. The Trigger Point Manual was used as the main reference for both palpation techniques and MTrP' diagnostic criteria (Simons et al., 1999).

A consensus on MTrP' diagnostic criteria and palpation procedures was established prior to the study. As proposed by Myburgh (Myburgh et al., 2011), a clinically relevant MTrP was defined as a taut band of muscle fibres, which when palpated, elicited either one or a combination of spot tenderness, pain recognition, or referred pain.

The methodology proposed for this study was developed according to the Quality Appraisal of Reliability Studies Checklist, proposed by Lucas et al. (Lucas et al., 2010b). It includes 11 items that cover seven different domains: the spectrum of subjects, the spectrum of examiners, examiner' blinding, the order' effects of examination, the suitability of the time interval between repeated measurements, appropriate test' application and interpretation, and finally, and appropriate statistical analysis (appendix IX).

4.3.1 Participants

A total of 33 volunteers who suffered from neck/shoulder pain, were enrolled for the screening phase, and they'd been derived from amongst students and employees of the San Raffaele Scientific Institute. Finally, 24 volunteers (23 female and 1 male; age 24 ± 3 years) with a least one MTrP in the upper

trapezius (left or right), participated in the study. Public announcements were posted on a few message boards at San Raffaele institute to identify the potential participants. During the enrolment phase, information regarding the study was provided. All subjects signed a written informed consent form before participating in the screening phase.

Volunteers were requested to fill a case form, including the following information: age, gender, weight, height, presence of pain in the neck and shoulder region, diagnosis for neurological disorders, diagnosis for rheumatic disorders, diagnosis for psychiatric disorders, and previous whiplash associated disorders. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared (Mei et al., 2002). All the information was self-reported; no measurements or tests were conducted to verify the anthropometric details, or the reported diagnosis.

The inclusion' criteria listed at least one painful active movement of the cervical spine, and at least one painful neck/shoulder event, in the last 4 weeks. The exclusion criteria included the following: (1) history of neurological or rheumatic disorders; (2) whiplash in the previous 6 months; (3) the presence of scars or moles in the area of the upper trapezius muscles; (4) pregnancy; (5) clinical depression; and (6), a body mass index of 30 or higher. The proposed exclusion criteria were applied to avoid minor and major adverse events due to MTrP palpation' procedures. A body mass index of lower then 30 (i.e. the threshold beyond which people are considered obese) was necessary, as the reliability and accuracy of MTrP' palpation is affected by the amount of the subcutaneous tissue.

In order to ensure unbiased enrolment, two physiotherapists carried out a screening procedure one day before the collection of data. The purpose of the screening procedure was to ensure the presence of at least one clinically relevant MTrP in either the left or right upper trapezius. To test the accuracy of the MTrP' palpation, the presence of at least one MTrP was assumed.

The two physiotherapists performed the palpatory assessment in two separate rooms, and without the possibility of knowing each other's judgment as to the presence of a MTrP. Subject' enrolment was confirmed when there was agreement between the two physiotherapists.

4.3.2 Procedures

All the enrolled subjects (n = 24), one day after the screening, met the experienced physiotherapist (the examiner) that had performed the palpation protocol, to locate the MTrPs. The study consisted of eight experimental sessions, with three subjects taking part in each one. Four sessions included subjects with MTrPs in the left, and four in the right upper trapezius. Sessions were arranged in two separate rooms, in order to avoid the subject's voice being recognised by the examiner. In the first room, one physiotherapist explained the experimental protocol and drew an anatomical landmark system (ALS) on the subject's shoulder using a surgical pen (figure 4.1).

The ALS consisted of a line between the acromial angle (AA) and the spinous process of the seventh cervical vertebra (C7). The distance between AA and C7 (ALS_d) was recorded for all of the subjects. In the second room, subjects were seated in front of the examiner, who was blindfolded and not allowed to speak. A sheet with pre-set answers was given to each subject and used to reply to the examiner's questions. This was considered necessary to ensure that the examiner didn't recognise subjects by their voice. Each subject was examined twice, and the MTrP detected during the first palpatory examination, was called MTrP_1, while the second MTrP, detected during the second session, was called MTrP_2. The subjects were allowed to rest for 10 minutes between the two consecutive examinations, which had been conducted in a randomized order. Before starting the palpation' procedures, the physiotherapist adjusted the sitting posture of the volunteers. The following aspects were checked: feet flat on the floor, knees bent at 90 degrees, lumbar spine and pelvis in neutral position, shoulders

back and relaxed, head in line with the chest, chin in, and back relaxed against the back of the chair.

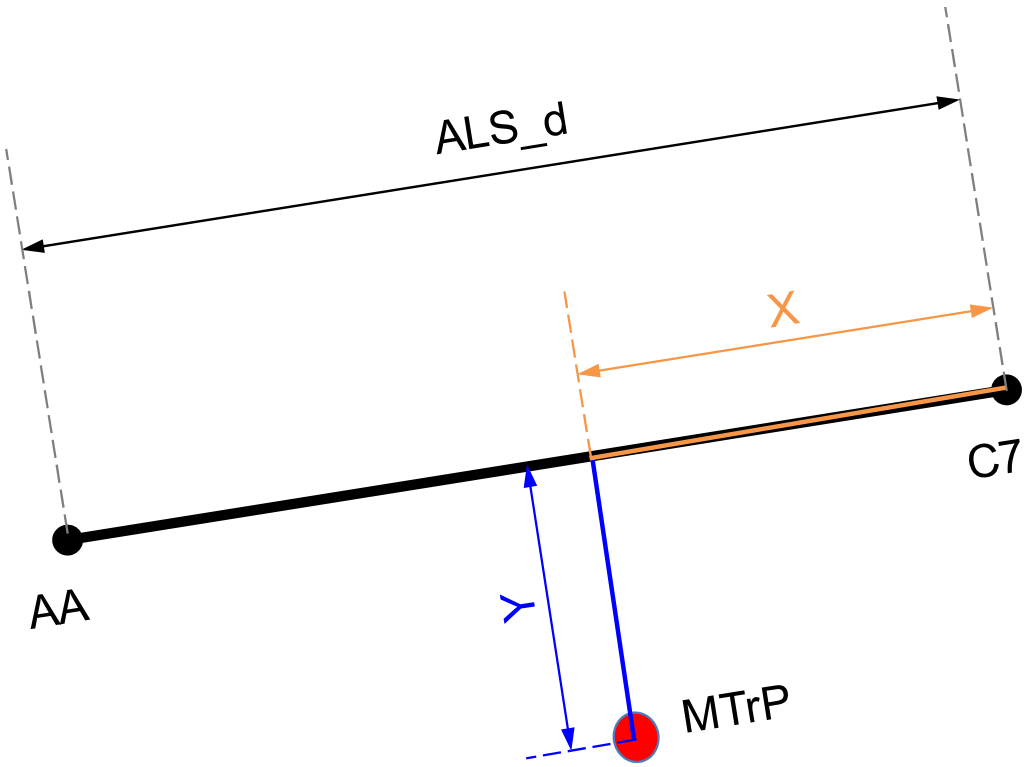

Figure 4.1: Anatomical landmark system. A graphical representation of the anatomical landmark system and the two variables, X and Y, used to define the MTrP' location on the upper trapezius. AA indicates the acromial angle of the scapula and C7 the spinal process of the seventh cervical vertebra. ALS_d is the distance between C7 and AA. MTrP, myofascial trigger point; AA, acriomial angle; C7, spinous process of the seventh vertebrae; ALS_d, distance between AA and C7.

4.3.3 Myofascial trigger point palpation protocol

Before each session, the examiner marked a point on the skin of the pad of his middle finger, using a surgical pen. The MTrPs were identified through a flat palpation technique using the index, the middle, and the ring finger of the

right hand. The spot tenderness identified on the taut band was used to define the location of the MTrPs on the upper trapezius muscle. Once the examiner confirmed the MTrP, location under the middle finger pad, the operator rolled the examiner's middle finger and marked on the skin, the contact between the point on the pad and the skin.

The palpation protocol was conducted according to the following steps:

1. Palpation over the upper trapezius region to identify one or more taut bands and their extension along the muscle fibres;
2. Gentle compression of contiguous spots along the detected taut band, in order to elicit pain and to locate accurately the spot tenderness. A positive reply from the subject to the question "Is this spot unusually painful?" was used to confirm the presence of spot tenderness. In case of more than one painful spot, the question "I will compress two spots, a first one and second one. Please tell me which is the most painful" was asked;
3. Perpendicular progressive and gentle compression on spot tenderness to elicit pain and verify the presence of pain recognition. A positive reply to the question " Do you recognize this pain as a familiar complaint?" was requested to confirm the presence of pain recognition;
4. Sustained painful compression (approximately 6 seconds) (Bennett, 2007) on spot tenderness was performed to elicit pain and verify the presence of referred pain. A positive reply to the question "Does the pain occur away from the spot that I am compressing? If yes, indicate where according to the anatomical regions reported on the sheet" was requested to confirm the presence of referred pain.

During all the palpation sessions, the examiner was seated and blindfolded. To answer the examiner's questions, the subjects were asked to point to the sheet with the pre-set answers (appendix X). The construction of the questions and potential answers had been agreed before the experimental

phase. The operator communicated the answers to the examiner. This indirect communication was necessary to prevent the subjects' recognition by the tone of their voice. A taut band, with at least one of the following, spot tenderness, pain recognition, or referred pain, was requested to confirm the MTrP' presence. If more than one spot tenderness was detected within the examined muscle, only the one that had elicited a familiar pain was considered (i.e. pain recognition). If a patient was not able to distinguish between two MTrPs in relation to his/her familiar pain, the examiner asked the subject to indicate which was the most painful. To avoid adverse effects due to tissue irritation, spot tenderness was compressed using the minimal force necessary to elicit pain in the subject.

4.3.4 Statistical analysis

The statistical analysis considered X and Y values in the two examinations, performed on the same subject. Intra-rater reliability was examined using intraclass correlation coefficients (ICC) (Chinn, 1990) and Bland–Altman plots (Bland and Altman, 1986, Bland and Altman, 1999), as they have been advocated to be the statistical methods of choice in reliability studies (Rankin and Stokes, 1998).

For the intra-rater reliability, considering two replicates of MTrP examination, a minimum sample size of 11 subjects was computed as being required, according to the method by Walter and collaborators (Walter et al., 1998). It was assumed an ICC value of at least 0.9, and it was determined that ICC values higher than 0.6 would be acceptable ($\alpha = 0.05$, $\beta = .020$) (Walter et al., 1998) (appendix XI). Since reliability of one examiner was analysed, a single measure $ICC_{(1,1)}$ model was applied (Rankin and Stokes, 1998). For the variables under consideration, the 95% confidence interval of the ICC values was computed. X and Y values were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$).

The criteria used for the interpretation of the ICCs were as follows: 0.00 – 0.25 indicated little or no correlation; 0.26 – 0.49 indicated low correlation; 0.50 –0.69 indicated moderate correlation; 0.70 – 0.89 indicated high correlation; and 0.90 – 1.00 indicated very high correlation (Munro, 2005). ICC values were not considered clinically useful if under 0.6 (Chinn, 1990). Bland–Altman plots were provided to give a visual representation of the size and range' differences between X and Y values.

Moreover, the distance between MTrP_1 and MTrP_2 was estimated (MTrPs_d) (figure 4.2). MTrP_d values were normally distributed for both left and right sides, as assessed by Shapiro-Wilk's test ($p > 0.05$). An Independent t-test was used to compare the MTrP_d in the left and right upper trapezius muscles. X scores, considering only the first MTrP examination (i.e. X1), were normally distributed for both left and right sides, as assessed by Shapiro-Wilk's test ($p > 0.05$); while Y values, considering only the first MTrP examination (i.e. Y2), were not normally distributedfor both left and right side, as assessed by Shapiro-Wilk's test ($p < 0.05$). A Mann-Whitney U test was used to determine if there were differences in X and Y values between left and right sides. Tests for normality are summarized in appendix XII. Statistical analyses were performed using SPSS Version 12.0 (SPSS Inc, Chicago, IL, USA). The significance level was set to $p < 0.05$ and CI was calculated at a confidence level of 95%.

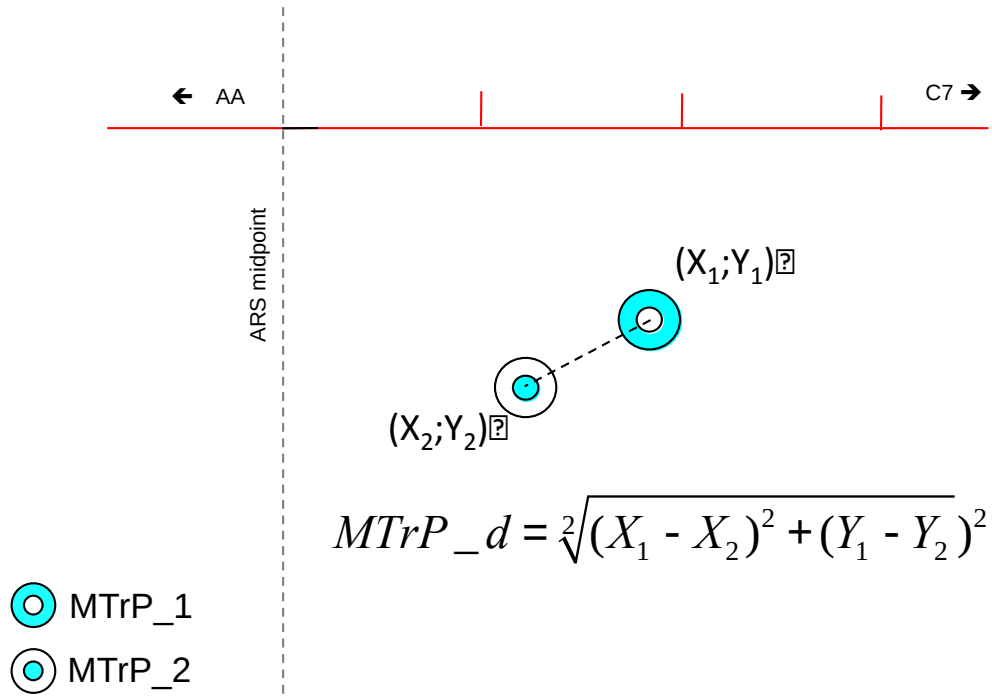

Figure 4.2: Formula to compute the distance between MTrP_1 and MTrP_2 (i.e. Pythagoras's theorem). MTrP_1, myofascial trigger point detected during the first palpatory examination; MTrP_2, myofascial trigger point detected during the second palpatory examination.

4.4 RESULTS

Characteristics of the enrolled volunteers are summarized in table 4.2. All study participants completed the palpation procedures, and the locations of MTrP_1 and MTrP_2 were recorded (table 4.3). Taut band, spot tenderness and pain recognition were identified in all subjects, while the referred pain was reported in nine subjects (appendix XIII). The referral zone was recorded according to the following anatomical region: head, neck, trunk, shoulder, arm, elbow, forearm and hand.

The ICC(1,1) values were 0.62 (95% CI: 0.30 – 0.81) for X, and 0.81 (95% CI: 0.61 – 0.91) for Y. The Bland–Altman plots are shown in figure 4.3 and figure 4.4. The values for mean difference were 0.04 mm for X, and -0.20

mm for Y. The standard deviation (SD) of the X value's differences was 13.4 mm and the 95% limits of agreement were -26.2 – 26.3 mm. The SD of Y value's differences was 13.6 mm and the 95% limits of agreement were -27.0 – 26.4 mm.

The mean MTrPs_d was 15 ± 11.0 mm, and, in 19 out of 24 cases, it was less than 20.0 mm (table 4.3 and appendix XIV). No statistically significant difference was found between the values of MTrP_d ($p = 0.52$) and between the X1 values ($p = 0.33$) of the left and right upper trapezius. However, a statistically significant difference was found for the Y1 values between the left and right upper trapezius ($P < 0.05$).

Table 4.2: Characteristics of the enrolled volunteers.

Subjects	Age(y)	Laterality		VAS	Pain frequency			Provocative movement
		L	R		<1 week	1 week	>1 week	
1	24		x	7			x	Flexion and left lateral bending
2	23		x	5			x	Flexion and left lateral bending
3	22		x	6	x			Left rotation + left lateral bending
4	22		x	3		x		Flexion + left lateral bending
5	22		x	5	x			Flexion + right rotation
6	22		x	3	x			Flexion + right lateral bending
7	22		x	6			x	Left lateral bending
8	23	x		6			x	Left lateral bending
9	30		x	5			x	Left rotation + left lateral bending
10	22		x	4		x		Extension and right rotation
11	22		x	3	x			Right lateral bending
12	23		x	3	x			Flexion

Table 4.2: Characteristics of the enrolled volunteers (continued).

13	22		x	6		x		Left rotation
14	26		x	6	x			Right rotation + right lateral bending
15	22		x	7	x			Flexion and right lateral bending
16	35		x	7			x	Left rotation
17	21		x	4			x	Right rotation
18	23		x	2		x		Flexion and right lateral bending
19	23		x	2		x		Flexion + right lateral bending
20	31		x	4			x	Left or right rotation and extension
21	25		x	6			x	Flexion and right lateral bending
22	22		x	4	x			Flexion + left lateral bending
23	22		x	4	x			Left lateral bending
24	25		x	4		x		Left rotation and left lateral bending
Mean(SD)	24(3)	1	23	4.7(1.5)	9	6	9	…

Table 4.3: MTrP' location (MTrP_1 and MTrP_2), with respect to the ALS, in the two examinations for each subject. Mean and standard deviation of the distance between MTrP_1 and MTrP_2 is reported in the last column (MTrPs_d). ALS_d indicates the distance between C7and the acromial angle. Abbreviations: MTrP, myofascial trigger point; ALS_d, distance between acromial angle and the spinous process of the seventh vertebrae; MTrP_1, myofascial trigger point detected during the first palpatory examination; MTrP_2, myofascial trigger point detected during the second palpatory examination; MTrPs_d, distance between MTrP_1 and MTrP_2.

Subjects	MTrP side	Session	ALS_d (mm)	MTrP_1		MTrP_2		MTrPs_d (mm)
				X (mm)	Y (mm)	X (mm)	Y (mm)	
1	R	1	214	64	75	51	62	18
2	R		210	69	70	70	70	1
3	R		216	89	32	86	34	4
4	L	2	210	90	8	78	22	18
5	L		192	62	74	67	90	17
6	L		195	75	7	80	9	5
7	R	3	185	95	32	76	32	19
8	R		205	75	35	81	40	8
9	R		195	60	56	76	25	35
10	R	4	220	115	35	80	68	48
11	R		215	78	57	75	65	9
12	R		215	96	39	94	40	2

Table 4.3: MTrP' location (MTrP_1 and MTrP_2), with respect to the ALS, in the two examinations for each subject. Mean and standard deviation of distance between MTrP_1 and MTrP_2 is reported in the last column (MTrPs_d). ALS_d indicates the distance between C7and the acromial angle. Abbreviations: MTrP, myofascial trigger point; ALS_d, distance between acromial angle and the spinous process of the seventh vertebrae; MTrP_1, myofascial trigger point detected during the first palpatory examination; MTrP_2, myofascial trigger point detected during the second palpatory examination; MTrPs_d, distance between MTrP_1 and MTrP_2 (Continued).

13	L		197	76	22	80	15	8
14	L	5	203	66	39	77	42	11
15	L		195	91	10	85	11	6
16	L		220	98	31	122	12	31
17	L	6	218	57	5	64	5	7
18	L		193	64	24	65	12	12
19	L		205	77	31	88	17	18
20	L	7	217	70	25	91	26	21
21	L		195	102	13	92	15	10
22	R		187	103	56	105	45	11
23	R	8	200	87	31	94	56	26
24	R		208	86	35	67	36	11
Mean(SD)	-	-	205(11)	81(16)	35(21)	81(15)	35(23)	15(11)

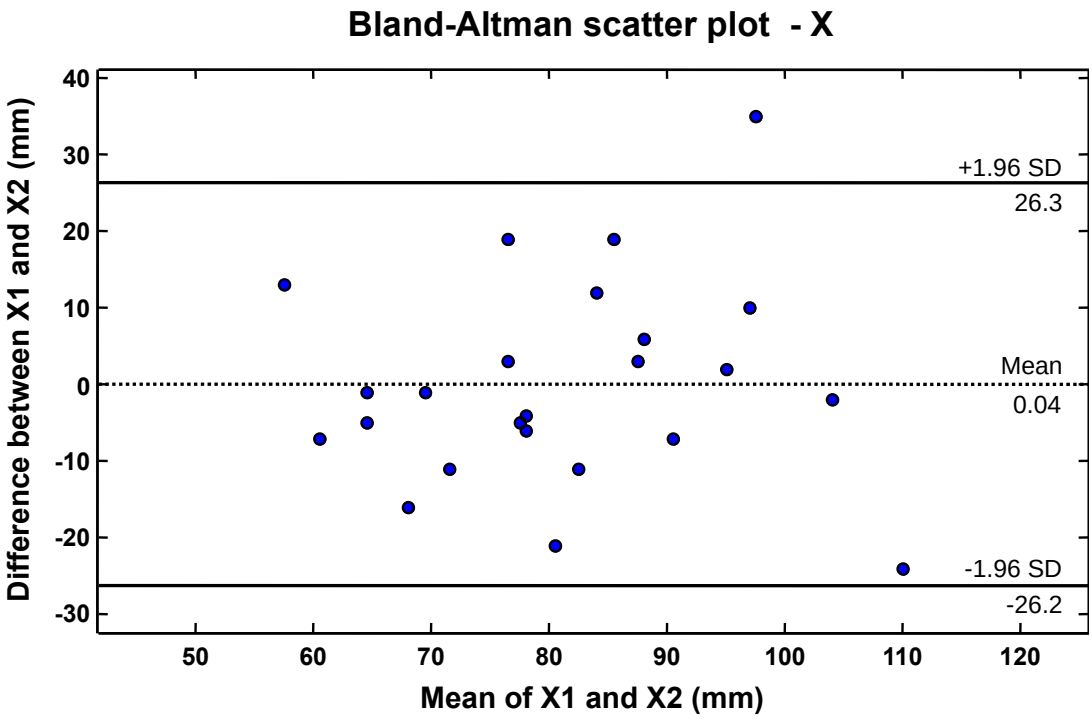

Figure 4.3: Bland-Altman plots showing the intra-rater reliability of the palpation protocol for locating MTrPs in the upper trapezius muscle. X is a variable used to define the MTrP' location according to the ALS. The difference of values is plotted against the mean values for each subject. The middle dotted line shows the mean of difference. The two lines above and below the mean of difference represent the 95% upper and lower limits (1.96 standard deviations). X1, X value measured during the first palpatory examination; X2, X value measured during the second palpatory examination.

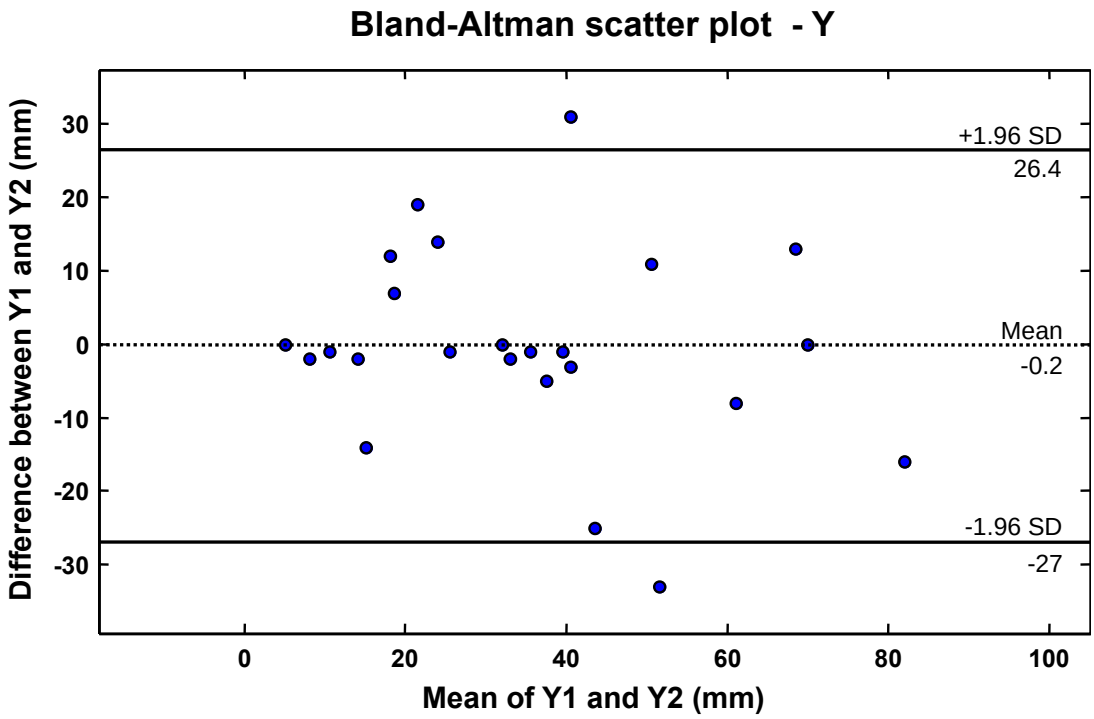

Figure 4.4: Bland-Altman plots showing the intra-rater reliability of the palpation protocol for locating MTrPs in the upper trapezius muscle. Y is a variable used to define the MTrP' location according to the ALS. The difference between values is plotted against the mean values for each subject. The middle dotted line shows the mean of difference. The two lines above and below the mean of difference represent the 95% upper and lower limits (1.96 standard deviations). Y1, Y value measured during the first palpatory examination; Y2, Y value measured during the second palpatory examination.

4.5 DISCUSSION

During the eight experimental sessions, 24 subjects with MTrPs were examined; 12 MTrPs were located in the left upper trapezius and 12 in the right upper trapezius. All subjects complained of at least one active painful movement of the cervical spine, and their VAS was 4.7 ± 1.5. Subjects were examined twice in the same session, and the MTrPs locations were defined by an operator measuring X and Y, according to ALS. No statistically significant difference was observed for the MTrP_d ($p = 0.52$) in the left or right upper trapezius, indicating that the particular side (left or right), does not affect the reliability of the palpation protocol.

The study' cohort showed that the MTrPs that had been located medially with respect to the ALS midpoint (figure 4.5), resulted in no statistically significant differences between the X values of the left and right upper trapezius muscles ($p = 0.32$). Interestingly, the Y values of the MTrPs showed a statistically significant difference between the left and right sides ($p < 0.05$). The MTrPs in the right upper trapezius were clearly located, as shown by the ALS, in the more caudal areas (figure 4.5). It is possible to speculate that this finding could be related to different muscular activities between the left and right upper limbs, as all the study subjects, except one, were right-handed. However, caution should be used in discussing the observed location asymmetry among the MTrPs, as morphological differences between left and right hemibody are frequently observed, and this can affect the comparability of the left and right ALS. This study' results showed a well-defined area for the MTrP' location in the upper trapezius region that had been similar to that described by Travell and Simons (1983) in their maps.

The ICC for the X and Y values did not fall under 0.6, suggesting a potential clinical application for the palpation protocol (Chinn, 1990). The reliability analysis showed a high correlation for Y ($ICC_{(1,1)} = 0.81$) and a moderate correlation for X ($ICC_{(1,1)} = 0.62$) (Munro, 2005).

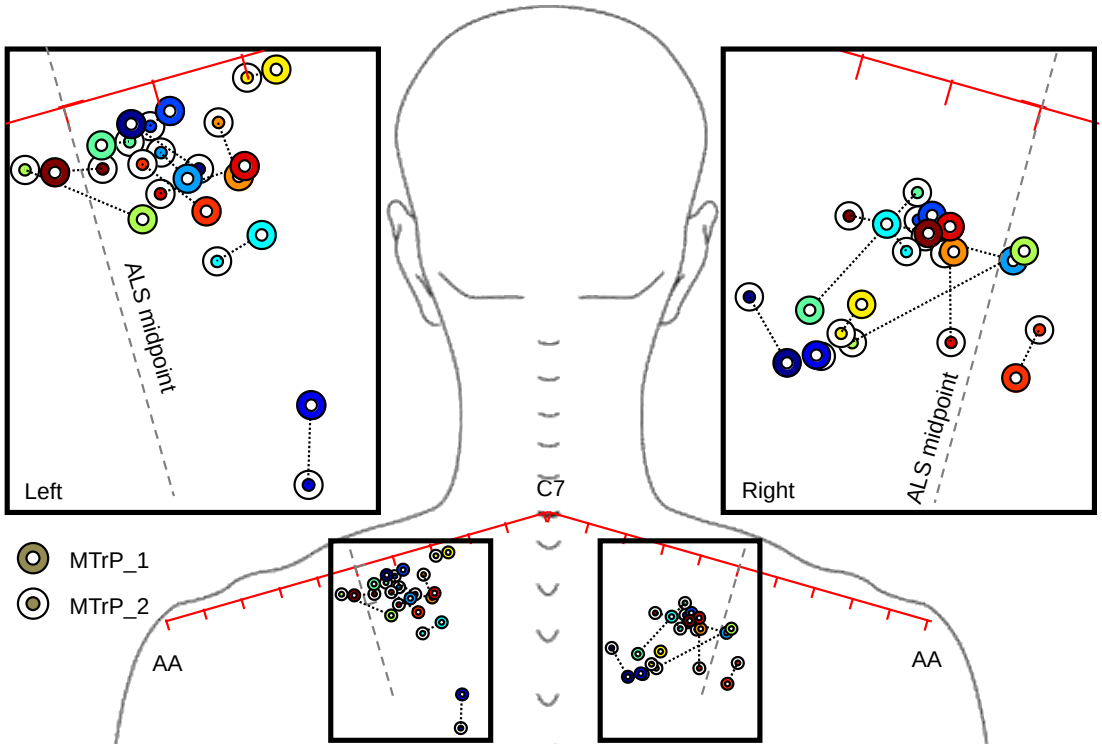

Figure 4.5: Locations of MTrP_1 and MTrP_2 for each subject according to the ALS. X and Y values are normalized with respect to ALS. Coloured circles are used to distinguish subjects in left and right ALS. The dotted line joining MTrP_1 and MTrP_2 represent the normalized MTrP_d. MTrPs are located in well-defined area medially to the ALS' midpoint, and right MTrPs are located in more caudal area ($p < 0.001$). ALS, anatomical landmark system; MTrP_1, myofascial trigger point detected during the first palpatory examination; MTrP_2, myofascial trigger point detected during the second palpatory examination; AA, acriomial angle; C7, spinous process of the seventh vertebrae; ALS, anatomical landmark system.

Considering the potential use of the palpation protocol in a clinical setting, further discussion is required concerning the difference that was found in the X and Y correlations. The Y values, according to the ALS, describe the vertical position of the MTrP, and are the first to be identified in accordance with the palpation protocol. The Y value can be used to detect the taut band in the upper trapezius muscle. The taut band is considered the only specific feature of the MTrP, and it is an objective sign. With regard to the muscle fibres direction, the indurated muscle bundles are palpated by snapping the bundles vertically. In the upper trapezius muscle, this procedure can be applied in optimal conditions, as the muscle fibres run approximately horizontal and parallel to the skin' surface (Johnson et al., 1994). A recent study (Sikdar et al., 2009), using an innovative ultrasound imaging technique, described the taut band in upper trapezius as an elliptical shape with a size of 0.16 ± 0.11 cm^2; thus, it can be reasonably palpated using fingers. A recent systematic review on the reliability of a physical examination for MTrP' diagnosis, reported kappa scores for the taut band ranging from -0.08 to 0.75 (Lucas et al., 2009). This inconsistent reliability was attributed to the anatomy of the different muscles and to the lack of appropriate training for the examiners. On the contrary, Myburgh and his collaborators stated the the spot tenderness, which has defined the MTrP' location, had a moderate evidence supporting its reliability within the upper trapezius (Myburgh 2008). Two studies (Gerwin et al 1997 and Lewoska et al.) that were included in a systematic review, reported kappa values reaching 0.6. It noted that Gerwin and his collaborators (Gerwin et al. 1997) had highlighted that the influence of a trained examiner has a critical role in increasing the reliability of the assessment of the location of the MTrP, during any examination.

The ICC scores for the X values, which indicate the horizontal location of the MTrPs, were considerably lower. The X values were defined by detecting the spot tenderness along the taut band, and they should be considered as a subjective sign that is related to the pain provocation of the palpation procedure. The spot tenderness of an MTrP is described as a highly

localized hyperalgesia on the taut band, but no investigations have been conducted to describe how pain threshold changes nearby to MTrP. Indeed, the question of whether hyperalgesia is localised, has never been addressed within a muscle harbouring a MTrP. It might be reasonable to speculate that a secondary hyperalgesia is presented nearby to MTrP, especially along the taut band, and that this makes difficult the manual localization of the spot tenderness. In addition, it was located by eliciting pain using the middle finger, which had a fingerprint on skin of approximately 1.5 cm^2. However, this potential error is limited by the palpation protocol that explicitly requires the examiner to elicit pain only by increasing pressure on the taut bands. Finally, the greater range of the X axis compared with that of the Y, could potentially result in a lower reliability. These factors could explain the difference in the reliability between the X and Y values.

The Bland–Altman plots support the reliability of the MTrP' palpation protocol by showing that the mean of the difference was close to zero for both X and Y. The limits of agreement were from 26.0 to -23.2 mm for X (figure 4.3) and from 26.2 to -29.6 mm for Y (figure 4.4). They indicated that both offered an acceptable error range and error size for the 24 MTrP' palpation procedures, especially if the area explored by the examiner is considered, an area that extended horizontality for 205 ± 11 mm.

In the examples where the results of Y are high, the Bland-Altman plot shows a lower reliability (figure 4.4). This suggests that palpation' reliability in the upper trapezius region decreases when compared with the inferiorly located MTrPs, though this remains speculative due to the small number of cases in this study. The clinical relevance of the observed error is limited, and the extent of error should not influence the use of standard treatment techniques that require the MTrP' location, such as ischemic compression, pressure release, strain counterstrain, ultrasound, or dry needling (Unalan et al., 2011, Montanez-Aguilera et al., 2010, Hsieh et al., 2007, Grieve et al., 2011).

The present study is the first to look at the intra-rater reliability of a palpation' protocol for locating an active MTrP in the upper trapezius region, and the only known study to follow the Quality Appraisal of Reliability Studies Checklist guidelines. The seven domains included in the checklist, were evaluated by considering the context for, and the aim of the current study. The coherence of the study's design was assured by the examiner being blinded to the clinical information and to any additional cues that could have influenced the outcomes. The spectrum of subjects for this study was intentionally limited to volunteers with MTrP in the upper trapezius who were without additional comorbidities. Moreover, only one examiner was included. The rationale for the latter was because this study has been designed specifically to estimate the reliability of the protocols delivered by the operator included within the scheduled cross-sectional study. It is acknowledged that this decision reduces the external validity of this study and limits the generalizability of the results. Nevertheless, similar results have been reported in a study focusing on the clinical precision among four clinicians, in detecting a latent MTrP in the trapezius muscle (Sciotti et al., 2001). The MTrP' location was estimated using a 3D optoelectronic system (OptoTRAK/320) and the overall magnitudes of the errors were 6.6 cm for the examiner 1, 4.6 cm for examiner 2, 3.8 cm for examiner 3 and 3.0 cm for the examiner 4. The precision was approximately 5 mm in the mediolateral, superoinferior, and anteroposterior directions. Generalizability of the results for this study's data was confirmed by the statistical analysis, although the reliability of the MTrP' location increased when the PPT was lower.

Finally, it is important to consider that the reliability of the palpation' protocol that has been investigatedl could be improved if applied in a clinical setting, where the examiner can benefit from visual contact, and be in direct verbal communication, with the patient. In order to prevent bias, this was not the case in current study.

4.6 LIMITATIONS OF THE STUDY

In the study on MTrP' palpation protocol, the relative reliability, defined as the degree of consistency to which measurements maintain their position over repeated measurements (Bruton et al., 2000), was computed using intra-class correlation coefficients (ICC). The absolute reliability, defined as degree to which repeated measurements vary for subjects, was analysed using the Bland-Altman plots (Bland and Altman, 1999, Bland and Altman, 1986).

For the ICC statistics, a threshold of 0.6 for clinical applicability (Chinn, 1990) was adopted, although ICC values between 0.50 and 0.69 indicate moderate correlation between measurements (Munro, 2005). This assumption can be questionable, especially when considering that the ICC value for X slightly exceeded the 0.6 threshold. Moreover, the 95% confidence interval ranged from 0.30 to 0.91. The upper and lower limits of agreement of the Bland-Altman plots were approximately 2.5 mm for both X and Y.

Moreover, a few methodological limitations need to be acknowledged. First, it is acknowledged that the pressure applied by the examiner on the spot tenderness of the taut band eliciting pain, was not measured. Thus, it was not possible to confirm that the subjects' responses to the painful stimuli, were due to the pressure on the spot tenderness, rather than to overpressure on a taut band point. Also, it cannot be excluded that the first palpatory examination of the MTrP may have influenced the sensitivity of the MTrP during the second examination. Although advisable, a PPT measurement of the spot tenderness was not feasible due to limitations within the experimental setup: the handling of an algometer was not possible as the examiner was blinded. Even in the case of having an additional operator, the PPT assessment, which includes three consecutive measurements, may increase the MTrP' sensibility due to the nociceptive repeated stimulation of the spot tenderness. PPT assessment could have been performed however with a single measurement (Grieve et al 2011; 2013).

Second, the possibility of a selection bias cannot be completely ruled out due to the procedure used for enrolling and screening of the study' subjects. Subjects were selected from volunteers with neck/shoulder complaints, and both operators had to agree on the presence of an MTrP before a patient could be enrolled. This procedure could have selected subjects with MTrPs showing a lower pain threshold; thus, it is not possible to compare this study' results with those from subjects having MTrPs with a higher pain threshold, such as that found with latent MTrPs. It was noticed that, on a few occasions, subjects reported a difficulty in comparing pain elicited by pressure applied to contiguous spots on the taut band. Also, this study's findings cannot be extended to subjects with different ages, or with a higher BMI. Similarly, it should be noted that most of the enrolled volunteers were female. Since females have lower pain thresholds and greater accuracy in discriminating pain sensations (Vallerand and Polomano, 2000, Mogil and Bailey, 2010), the same palpation' protocol used with a predominantly male population, on predominantly male population may yield different results. As for the IZ' reliability study, the upper trapezius muscle should be considered an optimal muscle to investigate the reliability for locating MTrPs. Indeed, its anatomical characteristics facilitate the manual palpation; it is a superficial muscle, it has parallel fibres, and usually it has a reduced thickness. Moreover, the criteria to diagnose the MTrP' presence in the upper trapezius muscle, showed an acceptable level of reliability when compared to other muscles (Lucas et al., 2009), and this is a critical requirement for investigating the reliability of locating MTrPs.

Furthermore, the results cannot be generalized to inexperienced physiotherapists and also the inter-rater reliability was not explored. Even in case of standardized training on MTrP' manual palpation, as it had been done in the current study, proper reliability cannot be assured.

4.7 CONCLUSIONS

The findings of this study suggest that an experienced physiotherapist, using the MTrP' palpation protocol, can reliably locate a MTrP in the upper trapezius muscle. Further research is required to investigate the inter-rater reliability of the protocol for MTrP' location, and to associate the current study' results both to different muscles and to physiotherapists with less experience.

CHAPTER 5

MYOFASCIAL TRIGGER POINTS AND INNERVATION ZONE LOCATIONS IN UPPER TRAPEZIUS MUSCLES

5.1 SUMMARY

Background: Myofascial trigger points (MTrPs) are hyperirritable spots located in taut bands of muscle fibres. Electrophysiological studies indicate that abnormal electrical activity is detectable near MTrPs. This phenomenon has been described as endplate noise and it has been purported to be associated MTrP pathophysiology. Thus, it is suggested that MTrPs may overlap the innervation zone (IZ). The purpose of this work was to describe the location of MTrPs and the IZ in the right upper trapezius.

Methods: Seventy-one individuals were screened and eventually, 24 subjects with neck pain and active MTrPs, and 24 subjects who were neck pain-free, with latent MTrPs, were enrolled. Surface electromyography (EMG) signals were detected using an electrode' matrix during isometric contraction of the upper trapezius. A physiotherapist examined the subject's trapezius to confirm the presence of MTrPs and to establish their location. The IZ' locations were identified by visual analysis of surface EMG signals. IZ' and MTrPs' locations were described using an anatomical landmark system (ALS), with the skin' area covered by the matrix, divided into four quadrants.

Results: No significant difference was observed between active and latent MTrPs' locations ($p = 0.6$). Forty-five MTrPs were in the third quadrant of the ALS, and 3 were included in the second quadrant. IZs were located approximately midway between the seventh cervical vertebrae and the acromial angle within a limited area between the second and third quadrants. The mean distance between the MTrP and the IZ was 10.4 ± 5.8 mm.

Conclusions: According to the these results, it was concluded that the IZ and the MTrPs are located in well-defined areas within the upper trapezius muscle. Moreover, MTrPs in upper trapezius are proximally located to the IZ but are not overlapping its location.

5.2 INTRODUCTION

MTrP' pathophysiology appears to be associated with the motor endplate zone (Hong and Simons, 1998, Kuan, 2009, Simons, 2001, Simons et al., 2002, Simons, 2008). This region, also known as the innervation zone (IZ), is where the α-motor neuron divides into a number of branches and synapses onto target muscle fibres. The IZ is usually described as being in the middle region of the muscle belly (Coërs and Woolf, 1959), but more complex IZ' spatial distributions have been reported in muscles with unipennate or multipennate fibre' arrangements (e.g. gastrocnemius and soleus) (Kim et al., 2005, Parratte et al., 2002, Prodanov et al., 2005). Needle electromyography (EMG)' studies have demonstrated that MTrPs contain minute loci producing characteristic low-amplitude electrical activity (Simons et al., 2002, Hubbard and Berkoff, 1993, Kuan et al., 2007) (i.e. active locus), and which is described in the literature as spontaneous electrical activity (SEA).

The origin of SEA has been extensively debated by experts. Initially, Hubbard and Berkoff attributed the source of SEA' action potentials to intrafusal muscle spindles located near MTrPs (Hubbard and Berkoff, 1993). Later, Simons considered previous work by Liley (Liley 1956), hypothesising that SEA originates from motor endplates and defined the latter as endplate noise (Simons, 2001). In support of the latter hypothesis, a needle EMG study showed that endplate noise was more prevalent in MTrPs than in adjacent sites (Simons et al., 2002).

This "motor endplate" hypothesis was also tested by Kuan et al., who injected MTrPs with botulinum toxin type A, to block acetylcholine release into the synaptic cleft, and found that the injection diminished SEA in MTrPs (Kuan et al., 2002).

Finally, the reported electrophysiological findings have been correlated with histological changes (Mense et al., 2003, Simons and Stolov, 1976) and local

biochemical alterations (Shah et al., 2008, Shah et al., 2005) (e.g. inflammatory mediators, neuropeptides, catecholamines, and cyokines) to support MTrP pathophysiology.

The reported experimental findings associated with MTrPs suggest that they are located close to the IZ (Mense et al., 2001, Kuan et al., 2007). In a research context aimed at clarifying the MTrP' aetiology, a confirmation of the overlapping between a MTrP and an IZ will help to clarify their interaction (i.e. the active locus and the sensitive locus) (Simons, 2004). Furthermore, manual identification of MTrPs could become useful to optimise treatments addressing to the IZ, such as botulinum injections for both cervical dystonia and myofascial pain (Soares et al., 2012, Delnooz and van de Warrenburg, 2012).

A technology, based on EMG' signals, and useful for detecting the IZ in vivo, has recently been proposed (Barbero et al., 2011, Masuda et al., 1983a, Merletti et al., 2003, Saitou et al., 2000), and to-date, no study has assessed both MTrP' and IZ' locations. Therefore, the purpose of this work was to describe MTrP' and IZ' locations in the upper trapezius muscle. The following hypothesis was tested in a clinical study: the distance between the IZ and the MTrPs in theupper trapezius, is different from zero.

5.3 METHODS

All experimental sessions were conducted between July and November 2008, at the laboratory of movement analysis of the San Raffaele Scientific Institute in Milan, Italy. The Internal Ethical Committee of the San Raffaele Scientific Institute and Research Ethics Committee of Queen Margaret University (Edinburgh) approved the protocol. All experiments were conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding of the subjects. The study

respected the Ethical Guidelines for Pain Research in Humans (Charlton, 2005).

Before commencement of the study, potential participants were fully informed about the goals, procedures and risks of the study, using an information sheet. All participants signed an informed consent form before enrolling in the study.

5.3.1 Participants

Twenty-nine patients with chronic mechanical neck pain, and 42 pain-free subjects with negative histories for neck and shoulder pain, were screened for participation in the study, according to the inclusion and exclusion criteria. Patients were recruited through the Rehabilitation Service of San Raffaele Hospital, and pain-free subjects consisted of San Raffaele Scientific Institute' employees. The healthy volunteers were recruited using a public announcement, posted at the DIBIT (Dipartimento di Biotecnologie). A convenience sampling was used to select the volunteers, due to fact that no previous studies were available on the primary outcome measure (i.e. the distance between the IZ and the MTrPs). This study should be considered a descriptive study, including a continuous variable, and the sample calculation ($n = 4Z\alpha^2S^2/W^2$, $Z\alpha = 1.96$) required three parameters: the confidence level (usually 95%), the desired width of the confidence interval (W) and the standard deviation of the investigated variables (S) (Hulley, 2001). Even assuming a width of the confidence interval, the standard deviation of the distance between the IZ and the MTrPs was not available.

Mechanical neck pain was defined as pain elicited by active cervical spine movements and perceived anywhere in the posterior region of the cervical spine, from the superior nuchal line to the first thoracic spinous process (Bogduk and McGuirk, 2006). Subjects with neck pain were screened, as they usually show MTrPs in the upper and mid trapezius muscles (Travell and Simons, 1983) .

Patients had to meet the following inclusion criteria: mechanical neck pain, neck pain' history lasting more than 3 months, and an active or latent MTrP in the right upper trapezius. Exclusion criteria were: positive history for neurological or rheumatic disorders, radiculopathy, fibromyalgia, joint disorders, whiplash in the previous 6 months, pregnancy, clinical depression and a body mass index ≥30. Concomitant painful disorders and psycho emotional distress were ruled out to avoid adverse effects and confounding factors during the MTrP' palpation procedure. This was done by a self-certification. Subjects with a BMI lower than 30, were excluded because an excessive subcutaneous fat layer thickness can limit the MTrP' palpation.

Fifty-two subjects were enrolled and eventually, 48 subjects were analysed: 18 patients with chronic neck pain and active MTrPs, 6 patients with chronic neck pain and latent MTrPs, and 24 pain-free subjects with latent MTrPs. The following subjects were excluded from the study:

- 16 subjects didn't show any latent or active MTrPs in the right upper trapezius muscle. Two out of 29 subjects with chronic mechanical neck pain, were negative to painful spot and taut band criteria. Fourteen out of 42 healthy subjects were negative to painful spot and taut band criteria.
- 3 subjects were excluded due to a positive history for neurological disorders
- 4 subjects showed an MTrP located outside the area covered by the electrode' matrix. Three out of 27 subjects were chronic neck pain with active MTrPs. One out of 24, was a healthy subject with a latent MTrP (figure 5.1).

During the screening phase, MTrPs were detected by a physiotherapist with 10 years of clinical experience specialising in myofascial pain syndrome' diagnosis and management. The physiotherapist explored the upper trapezius area using a flat palpation technique (Travell and Simons, 1983). Only neck pain patients with an active or latent MTrP, and pain-free subjects

with latent MTrPs, were considered for further analysis. Diagnostic criteria for an active MTrP were: the presence of a taut band within the upper trapezius muscle and at least one of the following clinical signs; the presence of a spot tenderness within the taut band, the reproduction of pain during mechanical stimulation of the spot tenderness, and the reproduction of a referred pain with mechanical stimulation of the spot tenderness (Myburgh et al., 2008). The presence of a local twitch response, which may occur during snapping palpation of the taut band, was not considered as one of the diagnostic criteria. Researchers have proposed the local twitch response as a confirmatory sign but its elicitation may require a vigorous palpation that is usually very painful (Simons, 1996). As its diagnostic values is not clearly defined, and it is not considered mandatory to diagnose the MTrP' presence, the assessor didn't elicit it specifically. This was also done to protect volunteers from harmful situations. Diagnostic criteria for latent MTrPs were the presence of both a taut band and spot tenderness.

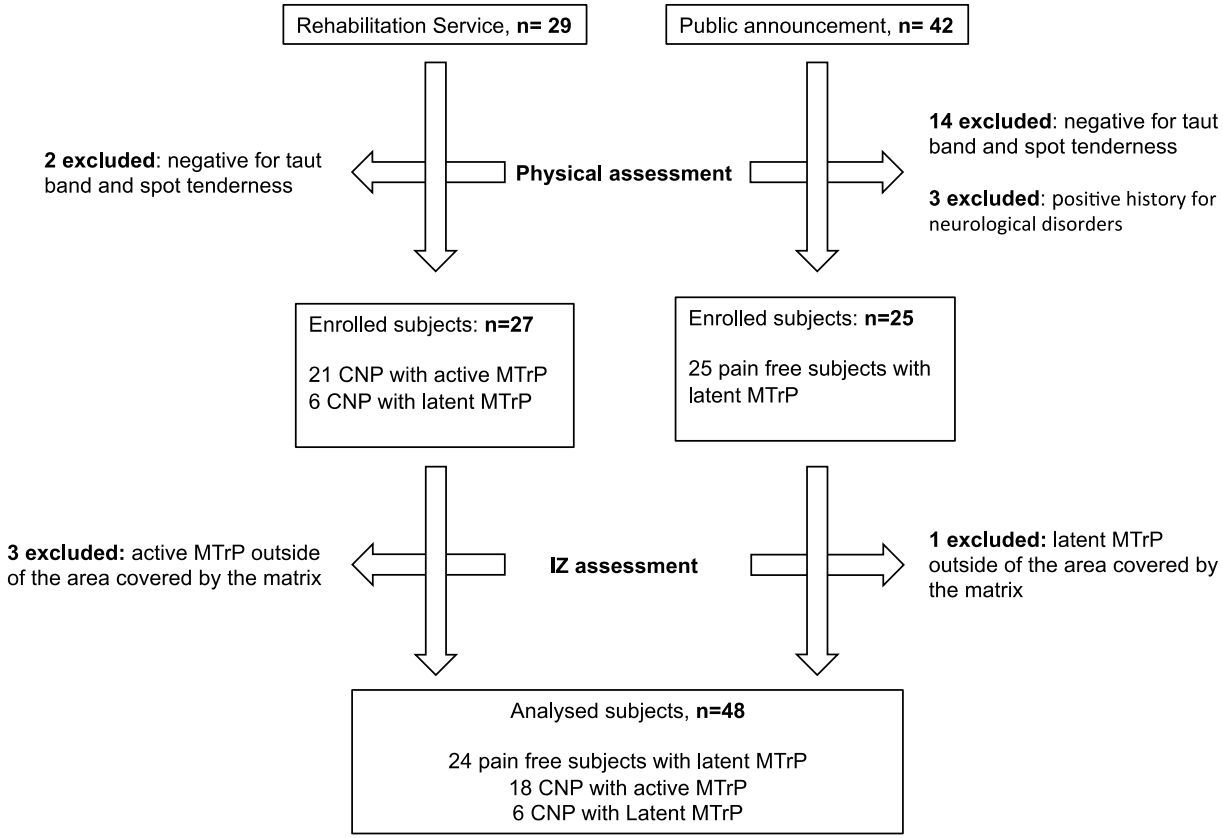

Figure 5.1: Flow diagram of volunteers' progress throughout the course of the study. Abbreviations: CNP, chronic neck pain; MTrP, myofascial trigger point.

5.3.2 Equipment

Surface electromyographic signals (sEMG) were detected using a matrix (model ELSCH064) composed by 4 columns of 13 electrodes and 1 column of 12, with 8 mm interelectrode distance (IED), designed by LISiN at Politecnico di Torino and manufactured by OT Bioelettronica, Torino, Italy.
The skin at the matrix site was shaved, lightly abraded using abrasive paste (Spes Medica srl, Genoa, Italy), and cleansed with isopropyl alcohol prior to the matrix application. The matrix was fixed on to the skin with double adhesive tape. The cavities corresponding with the electrodes, were filled with 20 µl conductive paste (TEN20, Weaver and Company, Aurora, Colorado, USA) using a spatula to ensure proper electrode–skin contact. Surface EMG signals were amplified with an EMG-USB amplifier (LISiN - OT Bioelettronica, Torino, Italy - bandwidth 10 – 750 Hz; adjustable gain between 500 and 10,000; sampling frequency 2048 Hz; 16 bits A/D converter). Samples were visualised on a laptop' screen during acquisition and stored on a personal computer OTBiolab software (OT Bioelettronica, Torino, Italy). During EMG experiments, the visualization of signals is important to exclude power-line' interference and artefacts.

In order to measure the torque exerted by the upper trapezius muscle, subjects were asked to sit on a custom designed chair and hold both chair handles (figure 5.2). The handle on the right side was fixed to a load cell in order to measure the force exerted during the shoulder elevation task. Subjects were asked to pull the handle toward the ceiling, without flexing their elbows and using 20% of their MVC. This contraction intensity was demonstrated to be appropriate to describe the IZ' location using surface EMG signals. Higher contraction intensity doesn't improve the estimation of the IZ' location, and is potentially painful if patients are involved (Barbero et al., 2011).

Force signals were acquired and amplified (band- width 0 – 80 Hz) using an MISOII amplifier (LISiN - OT Bioelettronica, Torino, Italy). Subject' feedback

was provided by a bar of light-emitting diodes indicating the percentage of the maximum voluntary contraction (MVC) that had been reached during each shoulder elevation. MTrP' pain pressure threshold (PPT) was assessed with a mechanical pressure algometer (Wagner Instruments, Greenwich, CT, USA). The application' rate was approximately 1 kg/s and the algometer was held perpendicular to the skin. The algometer had a rubber tip with a 1 cm^2 contact' area. PPT, defined as the minimum force applied at the MTrP' site which induces pain (Fischer, 1987), was measured three times on each site, and the average was recorded. The investigator instructed the subjects to stop the measurement by saying "ouch" as soon as a discernible sensation of pain was felt. The algometric measurement was performed while the subjects were seated on a chair with both the arms relaxed by the side.

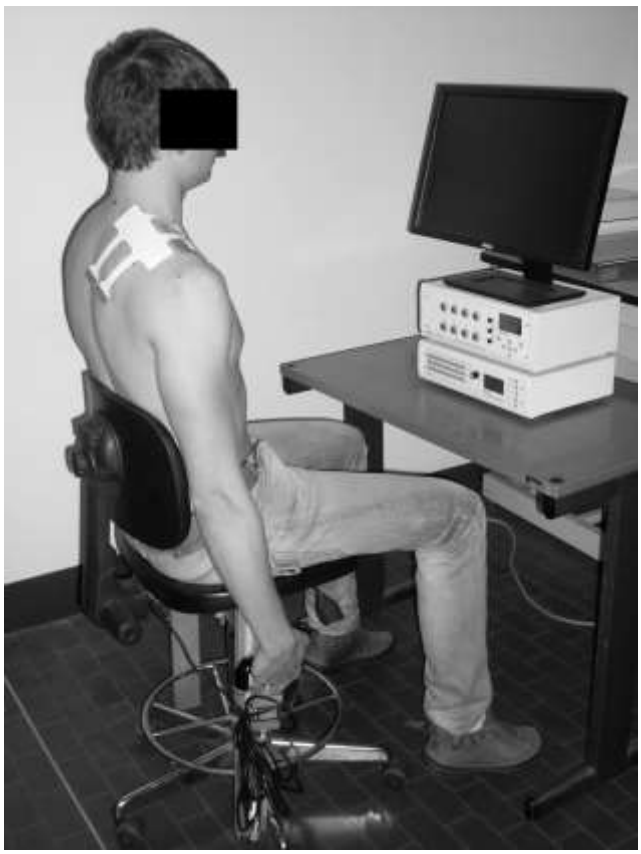

Figure 5.2: Experimental setup. Subjects sat in a custom made chair with a load cell connected to the right handle and were connected to an electrode' matrix (model ELSCH064, designed by LISiN at Politecnico di Torino and

manufactured by OT Bioelectronica, Torino, Italy), placed on the upper trapezius. They viewed the visual feedback device (MISO II, LISiN, OT Bioelettronica, Torino, Italy) during the experiment.

5.3.3 Procedures

The day after enrolment and immediately prior to the experimental procedure, patients with neck pain completed a Neck Disability Index (NDI) and a visual analogue scale (VAS). The NDI is a questionnaire, developed by Vernon and Mior, to assess self-rated disability in neck pain patients. It includes 10 items concerning pain and activities of daily living, including personal care, lifting, reading, headaches, concentration, work status, driving, sleeping and recreation. Its reliability and validity have been confirmed, and an Italian version (appendix XV) was validated recently (Monticone et al., 2012).

Scoring intervals for interpretation of the NDI were as follows: 0-4, no disability; 5-14, mild; 14-24, moderate; 25-34, severe; above 34, complete disability (Vernon and Mior, 1991). VAS is pain scale used to quantify pain' intensity. It consists of a 10 cm line drawn on a paper. It includes two written anchors, "the worst imaginable pain" and "no pain". The use of VAS has been validated for both chronic and experimental pain (Price et al., 1983).

In the current study, subjects reported their mean pain experienced in the last week was reported. Pain-free subjects started the experimental procedures immediately. Prior to each experimental session, the same operator used a surgical pen to draw a standardised anatomical landmark system (ALS) on the right shoulder of subjects, while they were seated on the data acquisition' chair. The ALS consisted of a line between the spinal process of the seventh vertebrae and the acromial angle (X-axis), and a second line perpendicular to the first one and passing through its midpoint (Y-axis). The centre of the electrode' matrix was placed on the intersection between the axes' with the matrix' columns parallel to the X-axis line, so that

the skin area covered by the matrix was divided into four quadrants (first, second, third and fourth).

To measure MVC, subjects were instructed to perform a shoulder elevation task by pulling the chair's handles upwards. The MVC' force level was determined as the maximum of three isometric contractions. Two minutes of rest were provided between the maximum exertions. Each subject was given instructions and allowed to practice for 5 minutes in order to learn the ability to keep the force level at 20% of MVC, using the feedback provided. Following this, surface EMG signals were acquired for 20 seconds at 20% MVC isometric contraction. After the electrode' matrix was removed, the expert physiotherapist performed an examination of the upper trapezius muscle using flat palpation techniques, in order to locate the MTrP according to the established diagnostic criteria (Myburgh et al., 2008). The latter's location was marked on the skin using a custom designed stamp (a $1cm^2$ circle with a dot in the centre). The dot in the centre was overlapped by the spot tenderness on the taut band, and its distance from the X- and Y-axes of the ALS was measured with a ruler. An operator blinded to the MTrP' location, performed IZ' visual analysis. The IZ was identified for each of the five columns of the bi-dimensional electrode' matrix by means of visual analysis of the single differential surface EMG signals (Merletti et al., 1999). The blinding of the operator was achieved by performing the visual analysis outwith the experimental session (i.e. offline), and while using signals that had records of the subject' and the experimental session' details purposely removed before analysis. The criteria to detect the MTrPs' location on each column were minimal amplitude and/or phase reversal of signals (Merletti et al., 2003, Masuda et al., 1983b). Subsequently, IZ' locations were described according to the ALS. To complete the IZ' location over the upper trapezius muscle IZs detected on matrix' columns were linked (linear interpolation) (figure 5.3).

The PPT over the stamp was assessed for each MTrP using a pressure algometer. PPT was measured three times while the subjects were seated

with the arms relaxed and positioned by the side of the subject's body. Measurements were promptly stopped by the subject saying " ouch". A pressure algometer has been suggested for the objective assessment of the MTrP, and its ability to measure MTrP with reliability and sensitivity, confirmed (Fischer, 1987, Reeves et al., 1986, Jaeger and Reeves, 1986). PPT, NDI (scale range, 0 to 50) and VAS (scale range, 0 to 100) provided detailed descriptions of the population that had been investigated.

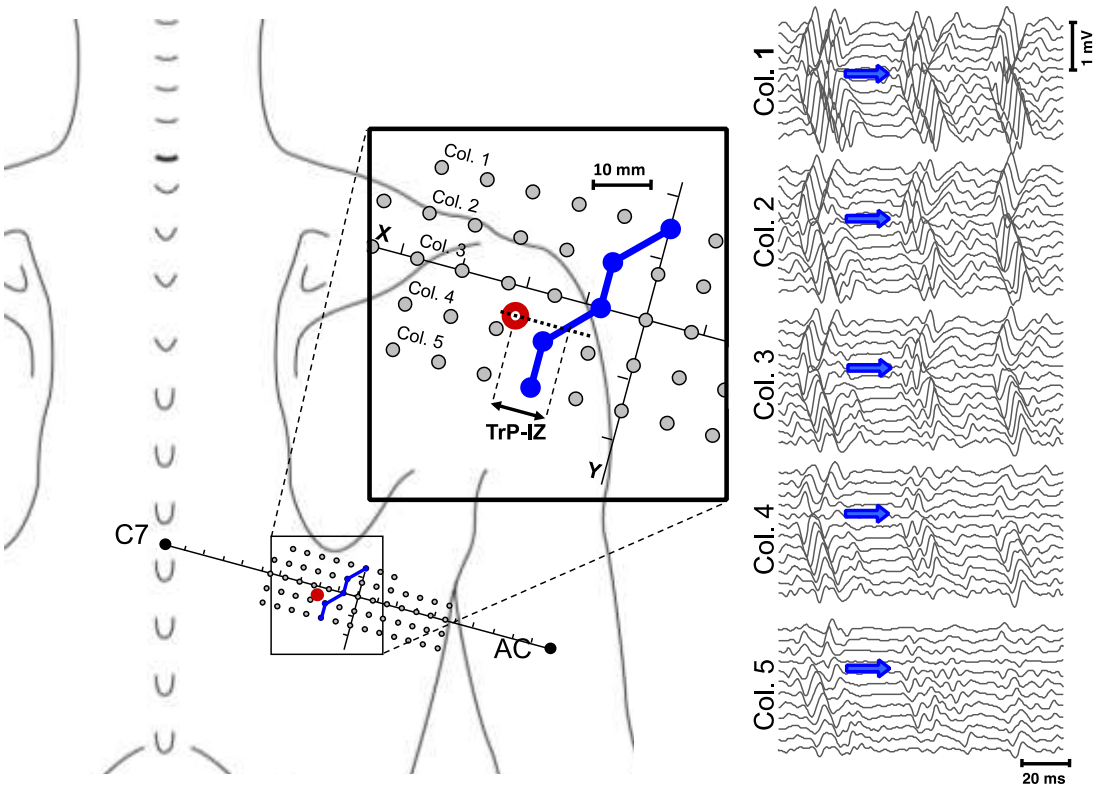

Figure 5.3: Electrode' matrix placement. The red dot indicates an MTrP in the upper trapezius and the blue line shows the IZ' location over the area covered by the electrode' matrix. The IZ was typically located medially to the Y-axis. TrP-IZ is the distance between spot tenderness in the MTrP' region and the IZ line. For each of the five columns of the matrix, EMG signals are reported and IZ' locations indicated using a blue arrow. The IZ' location was

detected where EMG signals showed minimal amplitude and/or phase reversal. AC, acromial angle; C7, spinous process of the seventh vertebrae

5.3.4 Statistical analysis

The distribution of the dependent variables was explored by using a histogram for each outcome measure. The sample size was small (< 50 samples), thus data were tested for Gaussian distribution using a Shapiro-Wilk test. The mean and standard deviation of NDI, VAS and MTrPs' PPTs were calculated in order to describe the subjects' clinical parameters. IZ' and MTrP' locations were described in accordance with the four quadrants that had been defined by the ALS. The distance between the spot tenderness and the IZ (TrP- IZ) was computed by tracing pathway parallel to the X-axis, reflecting the upper trapezius' fibre' direction (Johnson et al., 1994), and described as mean and SD. The TrP-IZ was measured along the X-axis because the intention was to intercept the IZs located in the same fibres that included the MTrP (figure 5.3). The correlations among variables (NDI, MTrPs' PPT, VAS, X, Y, TrP-IZ) were examined using Pearson' product-moment correlation coefficients. An independent t-test was used to compare the values of X, Y and TrP-IZ in active and latent MTrPs, and finally, to verify that the TrP-IZ was significantly different from zero. SPSS version 19 (SPSS Inc., IBM Company, New York, NY, USA) was used to perform the statistical analysis and the level of significance was set at 0.05, and each CI was calculated at a confidence level of 95%.

5.4 RESULTS

Variables' distribution was assessed by visual inspection of histograms (appendix XVI). All the variables showed a normal distribution, as assessed by Shapiro-Wilk's test ($p > 0.05$) (X, $p = 0.9$; Y, $p = 01$; TrP_IZ , $p = 0.68$; PTT, $p = 0.28$; NDI, $p = 0.07$; VAS, $p = 0.28$). Details of statistics for normality are summarized in the appendix XVI. The neck pain patients

showed a mean VAS score of 37.3 ± 15.2 and a mean NDI score of 11 ± 5 out 50. The overall mean PPT of the MTrPs was 2.6 ± 0.9 kg/cm^2 (table 5.1), while for healthy subjects showed a mean of 2.8 ± 0.8 kg/cm^2 and for neck pain patients showed a mean of 2.4 ± 0.9 kg/cm^2. No significant correlations were found among variables except between X and TrP-IZ ($p < 0.01$). According to the ALS, 45 subjects showed an MTrP which was medially located with respect to the Y-axis, and all the MTrPs were located in the third quadrant, except 3 that were located in second quadrant (figure 5.3). No statistically significant difference was found for X ($p = 0.6$) or Y ($p = 0.1$) values between active and latent MTrPs.

The IZ was successfully detected in all subjects, for each of the matrix' columns, and was not larger than 8 mm (i.e., the IED) (appendix XVII). Typically, the IZ was located medially with respect to the Y-axis and not further than 2.4 cm from the Y-axis (i.e., 3 IED) and for the most part, in an area that extended from the second to the third quadrant. It was occasionally included partially in the first and fourth quadrants (7 out of 48 subjects) (figure 5.4).

The mean TrP-IZ was 10.4 ± 5.8 mm, with no statistically significant difference between active and latent MTrPs ($p = 0.6$). TrP-IZ was significantly different from zero ($p <0.01$). figure 5.4 shows both MTrPs' and IZ' locations, according to the ALS.

Table 5.1: Summarised results. Column two indicates patient group: H, healthy group; NP, neck pain group. Column three indicates MTrP' status: A, active MTrP; L, latent MTrP. Neck disability index (NDI) was not requested (n/r) from healthy subjects.

Subjects	Group	MTrPs	VAS score	NDI score	PPT (Kg/cm$^{2)}$	X (mm)	Y (mm)	TrP-IZ (mm)
1	H	L	0	n/r	2.9	-1	-1	10
2	NP	A	51	21	1.3	-1	-1.5	2
3	NP	L	21	8	2.1	-0.6	-1.5	10.5
4	NP	A	30	7	3.9	-2.4	-1.6	4
5	H	L	0	n/r	5.2	-1.2	0	0
6	NP	A	49	18	2	-1.8	-1.3	14
7	H	L	0	n/r	2.5	-1.9	-0.8	3
8	H	L	0	n/r	3.7	-2	-0.6	16
9	H	L	0	n/r	1.9	-1.7	-0.3	14.5
10	NP	A	19	5	1.6	-2.5	-0.7	17.5
11	NP	A	41	8	1.5	-2.3	-1.2	11
12	NP	A	49	18	2.9	-1.3	-1.3	1
13	NP	A	39	15	1.8	-1.6	-0.6	9
14	NP	A	30	8	1	-2.7	-1.4	10

Table 5.1: Summarised results. Column two indicates patient group: H, healthy group; NP, neck pain group. Column three indicates MTrP' status: A, active MTrP; L, latent MTrP. (Continued)

15	NP	A	55	6	1.7	-1.4	-1.4	12
16	NP	A	21	10	2.4	-2.2	-0.3	18
17	H	L	0	n/r	2.4	-2.1	-0.5	19.5
18*	NP	A	-	-	-	-0.9	-2.1	-
19	H	L	0	n/r	3.1	-0.1	-1.5	6.5
20	NP	L	75	8	2.3	-1.8	-1.3	6
21	H	L	0	n/r	2.9	-2.9	-1.4	9
22	H	L	0	n/r	2.5	-1.6	-0.3	9
23	H	L	0	n/r	2.8	-3.1	0	7
24	NP	L	46	7	3.4	-1.7	-0.1	16.5
25	H	L	0	n/r	2.7	-1.5	0	11
26	H	L	0	n/r	1.8	-2.7	-0.6	16
27	H	L	0	n/r	1.6	-1.1	-0.6	3
28	H	L	0	n/r	2.7	-2	-0.4	14

* Subjects who showed an MTrP located outside the matrix, were excluded from the analysis

Table 5.1: Summarised results. Column two indicates patient group: H, healthy group; NP, neck pain group. Column three indicates MTrP' status: A, active MTrP; L, latent MTrP. (Continued)

29	H	L	0	n/r	4.8	-2.7	-0.7	19
30	H	L	0	n/r	2.6	-2.9	-1	20
31	H	L	0	n/r	2.5	-0.7	-0.5	0.5
32	NP	A	27	11	2.7	-2.2	0	10
33*	H	L	-	-	-	-1.4	-1.7	-
34	H	L	0	n/r	3.2	-1.4	-0.6	10
35	H	L	0	n/r	2.3	-1.4	-0.7	2.5
36	H	L	0	n/r	1.9	-2.7	-0.5	12.5
37	H	L	0	n/r	3.6	-2.1	0	13
38	H	L	0	n/r	2.1	-2	0.1	8
39	NP	A	20	4	3.2	-2.9	-0.7	17
40	H	L	0	n/r	2.3	-2.6	0	14
41	H	L	0	n/r	3.4	-1.5	-1.4	12
42	NP	A	67	16	2	-2.3	0.5	11

* Subjects who showed an MTrP located outside the matrix, were excluded from the analysis

Table 5.1: Summarised results. Column two indicates patient group: H, healthy group; NP, neck pain group. Column three indicates MTrP' status: A, active MTrP; L, latent MTrP. (Continued)

43	NP	A	39	16	1.7	-2.2	-0.6	15
44	NP	L	47	13	2.7	-1.4	-1.5	10.5
45	NP	A	34	9	2.2	-1.9	-1.5	8
46	NP	A	23	4	3.5	-0.5	0	1
47	NP	L	33	13	4	-1.5	-0.4	1
48	NP	A	34	11	3.8	-2.1	-0.6	16
49	NP	L	25	9	2.1	-2.7	0.5	20
50	NP	A	21	14	3	-1	-0.9	6
51*	NP	A	-	-	-	-2	-1.9	-
52*	NP	A	-	-	-	-1.8	-1.9	-
Mean ± SD			**37.3 ± 15.2**	**11 ± 5**	**2.6 ± 0.9**			**10.4 ± 5.8**

* Subjects who showed an MTrP located outside the matrix, were excluded from the analysis

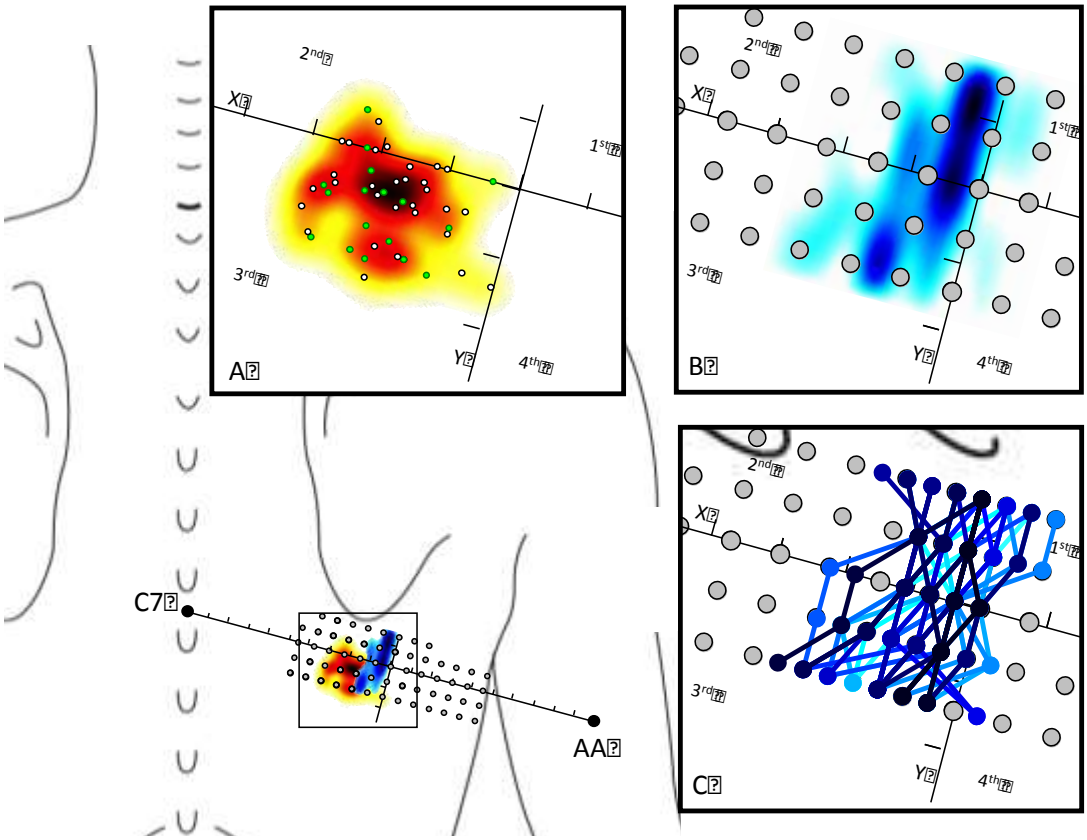

Figure 5.4: Graphical representation of MTrPs' and IZ' location in the upper trapezius according to the ALS in 48 subjects. A) Active MTrPs are represented as green circles and latent ones as white circles, and the colours indicate MTrP' spatial densities (a dark red spot indicates high trigger point' density). B) IZ' distribution, colour represents IZ' density (a dark blue area indicates high IZ' density). C) Each IZ' loction that is represented by a different colour, shows a linked IZ that had been detected within the columns of the matrix. AA, acromial angle; C7, spinous process of the seventh cervical vertebrae.

5.5 DISCUSSION

The purpose of this work was to describe the location of MTrPs and IZs in the upper trapezius muscle. Previous studies have reported that it is unclear whether the locations of the MTrPs and the IZs are closely related (Mense et al., 2001, Simons, 2004, Kuan et al., 2007). A study that investigates both these locations in the same subjects has not previously been undertaken. Previous studies have shown that the MTrP' region includes active loci, where it is possible to identify low-amplitude electrical activity (i.e., SEA), that had been attributed to motor endplate dysfunction, termed endplate noise. Additionally, high correlation between MTrP' site irritability and endplate noise' prevalence has been demonstrated by inserting an EMG needle (Kuan et al., 2007). The procedure for searching for endplate noise included the insertion of an intramuscular needle at the MTrP' region that had been identified during palpation (Simons et al., 2002). As stated by the authors, this suggests an immediate proximity between the MTrP and the IZ (Mense et al., 2001, Kuan et al., 2002, Kuan et al., 2007).

Results indicated that MTrPs were located in a well-defined area of the upper trapezius, with a PPT that was clearly lower than in normal muscles of healthy subjects (Fischer, 1987). For the upper trapezius muscle in healthy subjects without MTrPs, Fischer reported a mean PPT of 3.7 ± 1.9 kg/cm^2 for females, and 5.4 ± 2.8 kg/cm^2 for males. Moreover, the reported values are similar to those reported in previous studies including those for subjects with latent or active MTrPs (Sciotti et al., 2001, Simons et al., 2002, Kuan et al., 2002). In the current study, the area under investigation was the inferior part of upper trapezius and a portion of the upper part of the mid trapezius where fibres were described as being horizontally oriented (Johnson et al., 1994). This anatomical region is where patients with neck pain, typically report tenderness, and where both active and latent MTrPs are frequently observed (Fernandez-de-las-Penas et al., 2007). The data from the current study described an area for the MTrP' location in upper trapezius that is similar to

the MTrP' chart proposed by Simons and Travell (Travell and Simons, 1983). The latter approach produces results that match the results of the manual palpation study of this thesis, and which had used the same ALS (Barbero et al., 2012a). MTrPs in the upper trapezius appear to have a stereotyped location, and clinicians could use our ALS to guide their palpatory examinations.

No significant correlations were found among the variables, with the exception of those between X and TrP-IZ, which is an obvious finding considering how TrP-IZ had been computed. Additionally, no significant differences were observed for either X or Y values between active and latent MTrPs. A similar location for active and latent MTrPs would support, as described by Simons, a natural course for myofascial pain that includes a subclinical stage in which the MTrPs are not spontaneously painful (i.e., latent) (Simons, 1996). Forty-two neck pain-free subjects with a negative history for arm/shoulder complaints were screened, and just 16 of them were negative for taut bands with spot tenderness. The common presence of taut bands in pain-free subjects (Fernandez-de-las-Penas et al., 2007, Wolfe et al., 1992, Njoo and Van der Does, 1994) and the similar location for active and latent MTrPs, seem to suggest that taut bands could be considered as a necessary precursor to the development of a MTrP. It is likely that stress' factors (e.g. muscle overload or emotional distress) could be involved in the progression from latent to active (Simons, 1996). This was recently confirmed by Shah et al., who demonstrated that active and latent MTrPs contain the same biochemical substances (Shah et al., 2005) (bradykinin, substance P and serotonin), and that latters' concentration is lower in latent MTrPs compared with active MTrPs. In the current study, spot tenderness compression failed to evoke complaints (i.e. a negative pain recognition criterion) in just 6 of 24 subjects with neck pain, suggesting that MTrPs in the upper trapezius frequently contribute to neck pain. The presence of MTrPs should not be overlooked when examining subjects with painful conditions; moreover, high MTrP' prevalence has been reported in several selected

patient populations (Bron et al., 2011a, Alonso-Blanco et al., 2012, Itza et al., 2010, Skootsky et al., 1989) .

The electrode' matrix covered a 30.72 cm^2 area (9.6 cm × 3.2 cm), and the IZ can be approximately drawn on the skin as a straight line that runs orthogonally to the upper trapezius fibres and tends to curve medially towards the spine in its caudal part. A similar distribution for the IZ in the upper trapezius had been previously reported by Saito el al. in three healthy subjects (Saitou et al., 2000). However, this is the first time that the IZ' location was investigated in a large group of subjects, focusing on the area that extends over the muscle' surface. The same experimental setting was applied in a previous study that did not focus on the IZ' morphology (Barbero et al., 2011). It should be noted that there was limited variability for the IZ' location in the upper trapezius, and the current study's results support the generally accepted principle that muscles with parallel fibres contain IZs in the midbelly (Coërs and Woolf, 1959).

The current study's findings confirm that the MTrPs in the upper trapezius are located in the proximity of the IZ but do not overlap with it; rather, their locations are about 10 mm apart. In contrast with previous investigations distinct locations for IZs and MTrPs were observed, but it is important to note that this study had investigated investigated a different region of the upper trapezius, compared to those of previous studies. Interestingly, MTrPs were not equally distributed along the IZ and only affected specific groups of fibres in the upper trapezius muscle.

The close spatial relationship between IZ and MTrPs can be potentially useful to guide treatments targeting the IZ. As for example, the procedures for botulinum toxin injections in various pain conditions, including muscle spasticity, cervical dystonia, headache and myofascial pain (Soares et al., 2012, Delnooz and van de Warrenburg, 2012). A few limitations need to be taken into account when interpreting the results of this study. MTrP' locations were identified using spot tenderness located on taut bands by means of

palpation, in a similar way to previous studies (Simons et al., 2002, Kuan et al., 2007). Although this method has been shown to reliably locate MTrPs in the upper trapezius, it had been appreciated that this only provides an approximation of its location, ranging from a few millimetres to 1.5 centimetres (Sciotti et al., 2001).

5.6 LIMITATIONS OF THE STUDY

With respect to the cross-sectional study that reflects the analysis of data collected at one specific point in time, two aspects of the research design should be considered specifically. Firstly, the current study was carried out at one point in time and assessments at a different point in time may have provided different results. While it is not reasonable to expect a modification of the IZ' location, at least in the short or medium term, it is possible to speculate that the MTrP' spot tenderness varies its location during the clinical course of a MPS (Levin, 2006). Perpetuating factors or muscle' activations may have a role in this phenomenon. Thus, a prospective study may help not only in addressing the clinical course of a MTrP, but also to confirm its spatial relationship with the IZ. Secondly, in cross-sectional studies, it is hard to make causal inference for the observed variables (Levin, 2006). Any discussion, based on these results, on the underlying mechanism for the close spatial relationship between IZ and MTrP, will remain speculative.

Again, as for the reliability studies, the spatial relationship between IZ and MTrP was explored with reference to one muscle. The upper trapezius muscle has a unique fibre architecture and IZ' morphology, which can also heterogeneous amongst various peoples' trapezius muscles (Saitou et al., 2000). In muscles like the gastrocnemius (Kim et al., 2005), the IZ is scattered throughout the muscle, so the spatial relationship with hypothetical MTrPs could give different results.

Similarly, the possibility that MTrP' location in the upper trapezius depends on how its motor units are activated during functional activities, cannot be excluded. In muscles with a different spatial recruitment of motor units, the MTrPs' location may vary and thus, the spatial relationship between the MTrP and the IZ may be different.

A portion of the enrolled population (24 out of 48) were diagnosed to have had neck pain; which is a condition characterized by painful symptoms over the neck and shoulder region. In these patients, primary and secondary hyperalgesia may involve muscles, joints, and ligaments of this region; and manual palpation can elicit a painful response. This should be considered a potential confounding factor during the patient' manual examination aimed at identify MTrPs. Additionally, the possibility cannot be excluded that the MTrP' location is influenced or determined by the presence of the neck pain. Different medical conditions, which are prevalent in this anatomical region, may influence the MTrP' development, as well as modifying the MTrP's location within muscles.

Finally, it is important to note that the detection methodology for the IZs and MTrPs gives bi-dimensional locations on the skin, rather than a 3-dimensional location. Both the IZ and the MTrP are physically located within muscles by 3 dimensional coordinates. Alternative approaches, able to locate the IZ and the MTrP on a 3 dimensional coordinates system, may describe their spatial relationship better. The development of new imaging techniques will probably support these investigations.

5.7 CONCLUSIONS

MTrP' and IZ' locations were described according to the ALS in all enrolled subjects. MTrPs were located in well-defined areas of the upper trapezius, showing a typical location involving a mean TrP-IZ distance of 10 mm. MTrPs in the upper trapezius are proximally located to the IZ, but not overlapped by

it. These results provide an interesting insight for future research regarding the mechanism underlying the MTrP' iperalgesia. Moreover, the anatomical landmark system proposed in this study may help clinicians identify these areas.

CHAPTER 6

THESIS CONCLUSIONS

6.1 THESIS CONCLUSIONS

Muscle pain and tenderness in the absence of a clear diagnosis is a common clinical scenario for physiotherapists. Muscle pain can be localized or widespread, constant or intermittent, mild to severe and may reduce the patient's health-related quality of life (Fernandez-Perez et al., 2012, Cummings and Baldry, 2007). In these clinical circumstances, during the past century, as reported in chapter 1, several authors have described systematically what is now accepted as the MTrP (Harden et al., 2000). Clinicians of different medical disciplines are currently educated/trained to confirm the MPS diagnosis after the identification of at least one active MTrP (Charlton, 2005). Within the published literature, a precise set of diagnostic criteria has been recommended and the procedures for the manual examination of muscles, described (Tough et al., 2007). A narrative review published in 2015, stated that the gold standard for the MPS' diagnosis is the physical examination and the following criteria must be met: (1) palpation of the taut band; (2) identification of an exquisitely tender nodule in the taut band; and reproduction of the patient's symptomatic pain with sustained pressure (Shah et al., 2015). Nevertheless, a few limitations of the proposed physical examination should be acknowledged: training and palpation skills are critical, it is not applicable to all muscles, the characteristics of the physical examination's sensitivity and specificity are not available (Lucas et al., 2009). Moreover, there is a lack of consensus among clinicians regarding the physical findings associated with the MPS' diagnosis (Shah et al., 2015).

Estimates from the epidemiological studies, although generally of poor quality, suggest that MTrP is a common clinical feature; and it is frequently associated with the major musculoskeletal conditions such as for example, spinal conditions and shoulder pain (Lluch et al., 2015, Chiarotto et al., 2016, Fernandez-de-las-Penas et al., 2007, Iglesias-Gonzalez et al., 2013, Roach et al., 2013, Bron et al., 2011b). A search on the Physiotherapy Evidence Database (PEDro, http://www.pedro.org.au) using specific keywords for

MPS, clearly showed an increase of clinical studies for physiotherapy MTrP' treatment in the last decade (Figure 6.1) (Schneebeli et al., 2015). Clinical trials indicate a positive clinical response to different types of MTrP' treatments ranging from invasive to non-invasive, but recommendations from clinical guidelines are not available. However, according to the latest evidence from the literature, both dry needling and ischemic compression can be recommended for short and medium term pain relief (Cagnie et al., 2013, Kietrys et al., 2013).

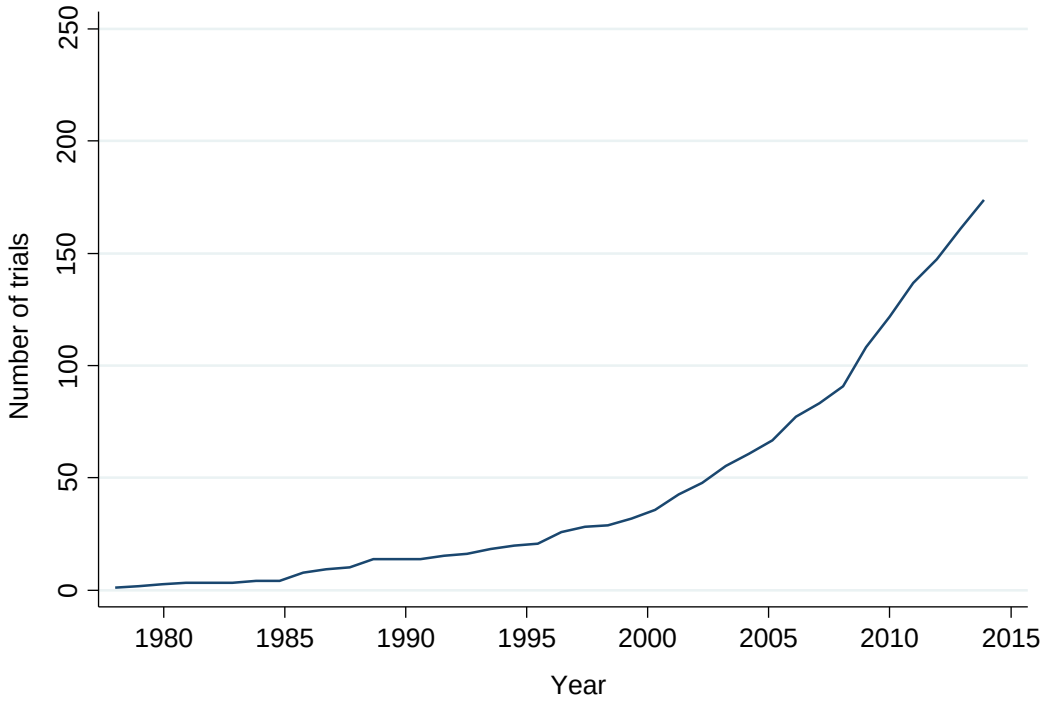

Figure 6.1: Cumulative number of randomized clinical trials and clinical studies on physiotherapy interventions for MTrP indexed in the Physiotherapy Evidence database (PEDro) by year, from 1978 to 2014. A total of 557 hits were retrieved, after screening for duplicates and inclusion criteria, 170 studies were included (Schneebeli et al. 2015).

A notable number of scientific papers have been published since 1942, when the first publication on MTrP by Janet Travell (1942) was accepted in Journal of the American Medical Association. Overall, three main research lines can be identified in the literature. MTrP aetiology; aimed at associating

pathophysiological elements like the endplate dysfunction, inflammatory mediators, or abnormal tissue properties, with the MTrP' presence. MTrP diagnostic procedures; aimed aimed at proposing a reliable and valid method to detect MTrPs. In this case, the final goal is to overcome the well-known limitations of the MTrP' physical examination based on manual palpation (Myburgh et al., 2011, Myburgh et al., 2008, Lucas et al., 2009). Lastly, MTrP' treatments; aimed at identifying the most effective treatments for the MTrPs. In this context, the priority is to provide evidence-based recommendations for invasive and non-invasive MTrP' treatment. Among the latter research' lines,

a high priority can be afforded towards investigating the aetiology of a MTrP. Investigating the contributions of end plate dysfunction to the latter would be particularly appropriate, since it is considered a central pathophysiological element of the integrated trigger point hypothesis, proposed by Simon et al (1999). The face validity of MTrPs is high and practitioners recognise its clinical relevance, but the validity of the MPS' diagnosis is discussed in the scientific community and controversies still exist (Johnson, 2002, Quintner et al., 2014, Quintner and Cohen, 1994). Quinter and his collaborators (2014) asserted that the MPS' construct should be considered a conjecture and that the derived hypothesis must be dismissed. Alternatively, they suggested that the MTrP' phenomena can be explained as secondary hyperalgesia of peripheral neural origin (Quintner et al., 2014). It has been argued that MTrPs are located nearby to the peripheral nerves, and their cause involves a focal inflammation of the nerve axons leading to ectopic impulse generation (Dilley and Bove, 2008, Dilley et al., 2005).

There is a need for further investigations focusing on specific pathophysiological elements regarding the MTrP. It is important to define what is the exact nature of this "enigmatic" clinical phenomenon (Simons, 2004). To strengthen the validity of the MPS' diagnosis, the MTrPs should be recognised as specific pain generators and the underlying pathophysiological features should be clearly defined. Nonspecific

symptoms like hyperalgesia or referred pain are not enough to distinguish MTrPs from other common musculoskeletal conditions. Any advances in this direction will help to establish the content validity of a MPS' diagnosis and thereafter, its relevance for clinical practice within the musculoskeletal field.

For this reason, the spatial relationship between MTrP and IZ was investigated. A central role of the endplates within MTrP' pathophysiology has been proposed (Mense et al., 2001). MTrP' location within muscles and its relation with respect to other anatomical structures, has been underestimated, although authors, as already discussed in previous chapters, advocated its proximity with the IZ. Interestingly, and also during clinical practice with patients complaining of muscle pain of the neck and shoulder region, it is noticeable that that MTrPs in certain muscles (i.e. upper trapezius, sternocleidomastoid muscle, teres major muscle, infraspinatus muscle) can be identified in a quite a defined location, within the muscle' belly.

This thesis involved the application of two procedures to conduct the experimental measurements. A first one was used to detect the IZ' location using surface EMG signals, and a second one to locate the MTrP using a manual palpation' procedure. Both the procedures were applied to the upper trapezius muscle.

The principal goal was to address the spatial relationship between IZ and MTrP, while acknowledging that the authors have proposed that MTrPs are located in the IZ (Kuan, 2009, Mense et al., 2001). Thus, evidence regarding the relative and the absolute reliability of the applied experimental procedures were considered important for meaningful discussion of the findings of the cross-sectional study investigating the IZ' and MTrP' locations. When the current evidence had been considered carefully, the proposed null hypothesis for the cross-sectional study was that the distance between the IZ

and the MTrP in the upper trapezius would not have been significantly different from zero.

The intra- and inter-rater reliability statistics indicated a high degree of agreement between repeated IZ' estimations. Kappa values ranged from 0.82 to 0.92, indicating that any trained operators can confidently apply the visual analysis of surface EMG to locate the IZ. Even in cases of uncertainty in the application of the visual analysis, the measurement' error in the estimation of the IZ' location did not exceed 4 mm (i.e. half IED). In the same study, the effect on IZ localization of the following factors: contraction' intensity, the selected EMG' epochs (i.e. acquisition period), the contraction' repetition and the matrix' repositioning. This was done to ensure that the IZ estimation was not affected by variables related to the EMG procedures for the acquisition of EMG signals. The measurement error corresponding to the disagreement between operators and expressed as a percentage, was higher in this case than that observed previously in the literature. However, in general, it again translated to a disagreement that was mostly within an error of 4 mm. This accuracy of 4 mm was considered suitable for the cross-sectional study. The reported results supported both relative and absolute reliability for the procedure to locate the IZ. In relation to the planned enrolment of neck pain patients, the low intensity contraction (i.e. 20% MVC) was considered optimal to define the IZ' location and to avoid painful contractions of the upper trapezius. It is well known that the number and size of motor units activated during a muscle contraction depends on the force magnitude (Henneman et al., 1965), but no information was available about the IZ' spatial distribution with respect to contraction intensity. In the upper trapezius muscle, the EMG signals collected during higher contraction' intensities indicated essentially the same IZ' location.

Subsequently in the cross-sectional study, IZ' locations were successfully identified using surface EMG. The analysis involved 48 subjects and included a total of 30 latent MTrPs and 18 active MTrPs. Both IZ' and MTrP' locations

were detected for all the enrolled subjects, and no statistically different location was detected for latent and active MTrPs. Interestingly, they were located in a well-defined area of the ALS (i.e. the third quadrant) that can be approximately described as the midfibre' region of the upper part of the mid trapezius muscle, or alternatively, of the inferior part of upper trapezius muscle (figure 5.4). A similar MTrP' distribution was also observed in the last intra-rater study on manual palpation. Although it is not possible to clearly define this anatomical area, the findings indicate the MTrPs were not uniformly distributed along the IZ of the upper trapezius. Their mean distance to the position of IZ was 10.4 ± 5.8 mm, with the IZ always medially located with respect to MTrP' spot tenderness. The distance between IZ and MTrP was significantly different from zero ($p < 0.01$), which means that the testing null hypothesis was rejected. According to the finding, it is not possible to assert a clear overlapping between the MTrP and the IZ, even though it was evident that MTrPs were proximally located to the IZ. Indeed, considering the two anatomical elements, it is not easy to define clearly what would be an overlapping; the IZ is a narrow band that runs vertically through the mid-belly region of the upper trapezius, and the MTrP isn't a well-defined area of hyperalgesia within a muscle. Moreover, the MTrP' entity has been located using the spot tenderness, which is an element that does not directly represent the physical dimension of MTrPs, and no investigation has been proposed on their relationship. The only preliminary evidence available on the physical dimension of the MTrP reported a cross sectional area of less than a square centimetre (Ballyns et al., 2011). The spot tenderness, that may be considered the peak of the MTrP' hyperalgesia, was located approximately one centimetre medial to the IZ. It is possible to affirm that the MTrP hyperalgesia, which is not limited to the spot tenderness, extended through the IZ' location. In support of our IZ' localization using sEMG, evidence that comes from a gross anatomical study on 22 cadavers described the IZ localization of trapezius muscle using a Sihler's Neural staining technique (Xie et al., 2015). Although the thesis' study of IZ' investigations were limited to the upper muscle, a visual analysis of the

findings from Xie et al. (2015) indicate very similar results to those of the current study (figure 6.2).

The reliability of the MTrP' palpation protocol was confirmed in the latter study (Barbero et al., 2012a), strengthening the findings of the cross-sectional study.

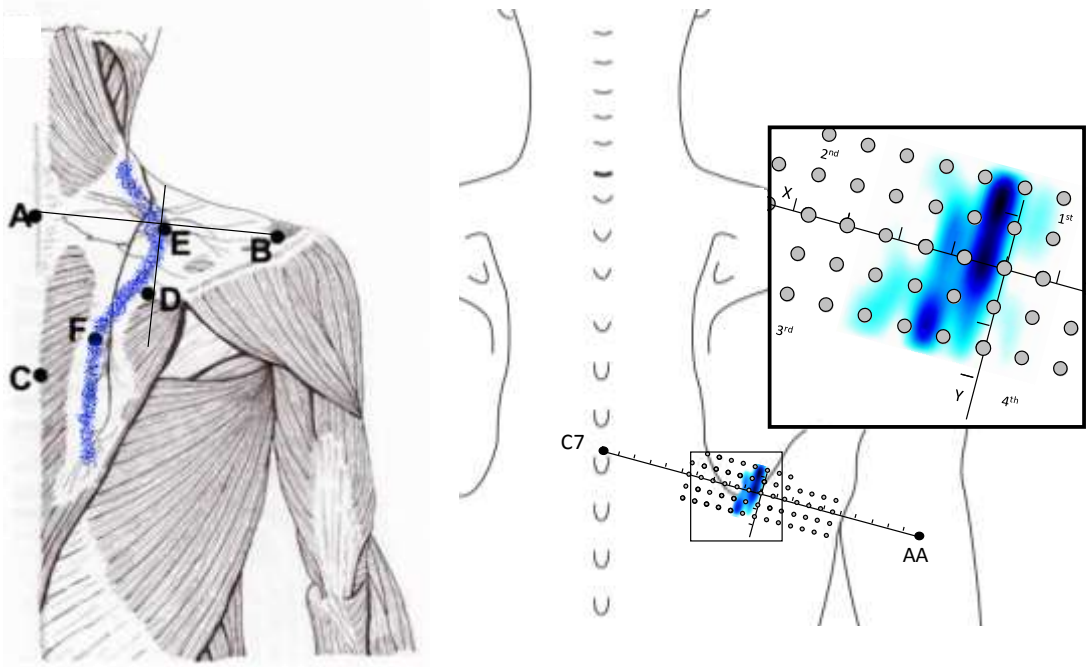

Figure 6.2: Two schematic diagrams of IZ' location of the upper trapezius muscle. The blue colour indicates the IZ. On the left side the IZ' location according to Xie et al. (2015), on right side, the IZ' location according to Barbero et al. (2013).

Specifically, the study was planned with the intention of producing evidence on the absolute and relative reliability of the manual procedures to locate MTrPs in the upper trapezius muscle. The ICC values showed from moderate to high correlation for the relevant outcome measures. Two variables, X and Y, expressed in millimetres, defined the MTrP, location. The mean distance between the MTrP' locations estimated in the two consecutive sessions, was 15.0 ±11.0 mm, with no difference between left and right

upper trapezius muscles. MTrPs in the left and the right upper trapezius muscles were located according to the ALS previously used for the study investigating IZ' reliability. MTrPs in the upper trapezius muscles on both sides of the body were located in well-defined area, medially to the ALS_d midpoint, and caudally with respect to line between acromial angle and the seventh cervical vertebra. This MTrP' spatial distribution in the upper trapezius was very similar to the one observed in the previous cross-sectional study. ICC values were 0.62 for the horizontal direction (x axis of the ALS) and 0.81 for the vertical direction (y axis of the ALS). Both the values reached the ICC threshold of 0.6, suggested as offering acceptable reliability for any clinical procedure or tool (Chinn, 1990). Nevertheless, the lower ICC value for the horizontal direction was noted. A difficulty in locating the spot tenderness along the taut band using palpation, was also reported by the examiner. Two elements were taken in account to explain low ICC values for the horizontal direction: firstly, the spot tenderness, which is a focal hyperalgesia, may involve an extended area and not just a discrete point on the ALS; secondly, the fingerprint used to explore the taut band has an area of approximately 1.5 cm^2, again relatively bigger than a discrete point on the ALS. However, the Bland-Altman plots showed that in both the directions a clinically acceptable error range, and additionally, the mean of the difference for repeated X and Y outcome measures were close to zero.

In summary, the results support the statements of a few leading researchers in suggesting that MTrPs are always located in the endplate zone. Interestingly, two recent papers (Fernandez-de-las-Penas and Dommerholt, 2014, Dommerholt and Gerwin, 2015), aimed at reviewing the MTrP' physiopathology and supporting the integrated trigger point hypothesis, cited our findings, to provide evidence that MTrPs are located proximal to the innervation zone (Barbero et al., 2013), and to refute an alternative pathophysiological hypothesis that had been formulated by Quinter al (Quintner and Cohen, 1994). The latter author claimed that the MTrP' construct lacks internal validity and is a tautological concept based on

empirical treatments of MPS (Quintner et al., 2014). Quinter suggested that myofascial pain is actually a nerve trunk pain, which is indeed, a specific kind of somatic referred pain (Quintner and Cohen, 1994). Local inflammation of the peripheral nerves associated with axonal mechanical sensitivity and spontaneous discharge of the nociceptors, was proposed as the alternative underlying pathophysiology (Quintner et al., 2014). Such a theory would imply a spatial localization of the MTrP, nearby to the nerve branches. If the anatomy of the upper trapezius was considered, many anastomosing branches can be found in all the muscle bellies, with a different distribution relative to the IZ. They originate from the spinal accessory nerve and from the trapezius branches of the cervical plexus, and form an S-shaped IZ throughout the muscle (figure 5.6) (Xie et al., 2015). This would imply theoretically, that MTrPs can be located anywhere in the muscle. The findings of the cross-sectional study and of the manual palpation' study, do not support such a spatial distribution and conversely, indicate for MTrPs a well-defined location within the upper trapezius muscle.

Finally, it is important to remark that the cross-sectional study explored the location of the IZ and of the MTrP at one specific point in time, without investigating directly the MTrP' physiopathology. An underling mechanism for the observed close spatial relationship between MTrP and IZ cannot be proposed, although the endplates' dysfunction remains a reasonable element of the integrated trigger point hypothesis (Mense et al., 2001).

Limitations associated with each of the thesis' studies have been detailed within the previous chapters. However, in order to avoid potential misinterpretations or generalizations of the findings, it may be useful to summarize here, the most important constraints, and to add a few more general considerations about the whole of the research project. Both IZ and MTrP have been examined only in upper trapezius muscle, and MTrPs have been identified only in subjects with neck pain. Thus, it is not possible to exclude the possibility that the spatial relationship between IZ and MTrP

might differ from that observed in the upper trapezius, when other muscles are considered. IZ' spatial distribution, as well as MTrP' spatial distribution, may have different features among different muscles. IZ' location and shape can vary substantially among muscles, according to their architecture (Beretta Piccoli et al., 2014). Similarly, it can be speculated that the observed stereotypical location of MTrPs in the upper trapezius may differ according to different muscle' functions (i.e. muscle activation patterns). Also, it is plausible that the MTrP' locations depend on the pathophysiology of the associated clinical conditions. For example, painful conditions of the cervical spine, such as chronic neck pain, whiplash associated disorders or cervical radiculopathy, can be characterized by different pain mechanisms or by different anatomical impairments (Bogduk, 2011b, Bogduk, 2009, Bogduk, 2011a). Currently, no information is available on how a MTrP in a specific muscle, is located according to different clinical conditions, but involving the same anatomical region.

The possibility that conditioning of the principal investigator might have influenced the thesis' findings exists; researcher bias that may limit the internal validity of the studies should be considered. During the three experimental studies, IZ' and MTrP' localization had been performed by the same investigator. The experimental procedures and data analysis conducted during the first study (i.e. the reliability of sEMG in locating the IZ) may have influenced the investigator during the second one (i.e. the cross-sectional study on the spatial relationship between IZ and MTrP). For the same reason, the second study may have influenced the investigator during the third study (i.e. the reliability of manual palpation in locating the MTrP in the upper trapezius muscle). Even though the investigator was blind to the subject during both IZ' and MTrP' localization, the possibility of an experimenter' bias can't be excluded. Lastly, as already mentioned in chapter 2, the original research' planning had been modified out of necessity. The study on the reliability of manual palpation in locating the MTrP was conducted after the cross-sectional study. This amendment to the original

research planning hasn't directly impacted on the research project because the research design and the experimental procedures of the cross-sectional study had not been constrained by the findings of the previous studies. Nevertheless, it should be acknowledged that the manual palpation protocol had been applied within the cross-sectional study, without information about its reliability having been available. Additionally, the investigator didn’t have the possibility of having performed the palpation protocol in an experimental context before the cross-sectional study had been initiated. The original planning should be considered optimal because in turn, it would have facilitated optimisation of the palpation protocol. The studies in the thesis had focused attention on experimental designs that had been group-based. Nevertheless, when focusing on clinical applications involving single-patient care, in the case of low reliability for the MTrP’ palpation protocol, it would not have been possible to either confirm the results of the cross-sectional study, or to have modified its experimental procedures to properly incorporate alterations to measurement' precision for use in case-study' applications. It is plausible experimentally that if the original planning for the studies' sequencing had been respected, different results could have been obtained.

CHAPTER 7

AREAS OF FUTURE RESEARCH

7.1 AREAS OF FUTURE RESEARCH

The findings of this thesis contribute to extending the body of knowledge regarding MTrPs. A close spatial relationship between MTrP and IZ was supported in the upper trapezius muscle as previously proposed (Kuan et al., 2007, Simons, 2001, Simons et al., 2002). However, generalization to other muscles should be avoided. Experimental observations from the current thesis' studies, as well as those from previous studies in the literature, should be limited to the upper trapezius muscle (Simons et al., 2002, Kuan et al., 2007). Future studies on this topic should include more than one muscle and especially, muscles with different fibres' architecture. Investigations such as these, will also clarify if MTrPs have a stereotypical location within the muscles, and will permit enhancement of the MTrP' charts proposed by Simons and Travell (Simons et al., 1999), in which the MTrP' location had not been systematically investigated. This information would be of help to clinicians during the manual examination of patients. The use of ALS for each muscle could also provide a useful guide for the palpation' procedures during clinical practice. Importantly, new research questions can be formulated in relation to our results, and other original studies may be proposed.

In the reliability study, and also in the study cross-sectional study, MTrPs were observed within a well-defined area of the upper trapezius. This region includes a limited number of fibres and a specific population of motor units of upper trapezius muscle. A study that had evaluated people with latent MTrPs in the upper trapezius muscle, documented early myoelectric manifestations of fatigue of the upper trapezius during sustained isometric contractions, but notably the muscle fibres close to the latent MTrP exhibited an anticipated and significant increase in surface EMG' amplitude (Ge et al., 2012). An increase of the intramuscular EMG' amplitude of the trapezius muscle has also been observed in subjects with latent MTrPs during synergistic muscle activation (Ge et al., 2014). The influences of MTrPs on motor function need

to be clarified. It may excite or inhibit normal motor control in their muscle of origin, or in functionally-related muscle.

Based on these observations, it may be suspected that the location of the peak myoelectrical activity of the upper trapezius muscle would be different in people with MTrPs, and that the peak activity would be located at the site of the MTrP. During a task, localized amplitude peaks correspond to activity of a motor unit pool whose territory is limited to region of the muscle (Vieira et al., 2011) and a spatial modification of this phenomenon may reflect an adaptation' strategy of the motor control system.

Novel high-density, two-dimensional surface EMG provides a measure of the electric potential distribution over a large surface area during muscle contraction (Gallina et al., 2013, Zwarts and Stegeman, 2003). Unlike classic bipolar EMG applications, this method provides a topographical representation of EMG amplitude, and can identify relative adaptations in the intensity of activity within regions of a muscle, and the location of the peak EMG amplitude across a large region of the muscle. High-density EMG studies have confirmed that either acute experimental muscle pain (Falla et al., 2009) or chronic clinical pain (Falla et al., 2010) may alter the distribution of muscle activity, and may cause a shift of the peak muscle activity. Considering these findings, it may be speculated that a long-lasting nociceptive irritant, such as a MTrP, could induce a spatial reorganization of muscle activity, although this prospect hasn't yet been investigated.

A study to extract topographical maps of surface EMG' amplitude to evaluate the location of peak muscle activity, may be proposed in subjects with and without MTrPs in the upper trapezius. Moreover, the relationship between the location of the MTrPs (i.e spot tenderness site) and the location of the peak EMG amplitude could be examined within this type of study. Investigations such as these, will help to unravel if MTrPs have an influence on motor control, at least at the level of the involved muscle. Additionally, this type of

study could provide evidence on the location of potential physiological impairments in relation to MTrP' spot tenderness.

The accuracy and the reliability of sEMG in locating the IZ, although limited to muscles with parallel fibres, has been demonstrated in the study. sEMG investigations are inexpensive and non-invasive, and no adverse events been reported during their applications. IZ' identification is a fast procedure and can be easily performed in a clinical setting. On the contrary, manual palpation to detect MTrPs showed a few limitations; its reliability is muscle dependent, it is operator-dependent, and it can't be considered a fully objective approach to measurement (Myburgh et al., 2008, Lucas et al., 2009, Tough et al., 2007). For these reasons, some research groups during the last few decades, have attempted to identify MTrPs using objective imaging techniques. Both ultrasound and magnetic resonance imaging have been proposed, and different imaging processing explored (Turo et al., 2015, Sikdar et al., 2009, Shankar and Reddy, 2012, Thomas and Shankar, 2013, Chen et al., 2007, Chen et al., 2008). Theoretical advantages can be attributed to the proposed imaging techniques: 1) they can be applied to all the muscles, 2) the MTrP can be objectively located within the muscle belly, 3) the MTrP' position can be established using a 3 dimensional approach. Moreover, in the case of magnetic resonance imaging, the taut band' stiffness may be estimated (Chen et al., 2007, Chen et al., 2008).

These imaging techniques can be proposed to further explore the spatial relationship between the IZ and the MTrP. For example, the combined applications of sEMG and ultrasound in locating MTrPs and IZs, would have overcome some constraints related to the MTrP' manual palpation. Additionally, in such a study, there would be the possibility to assess more than one muscle. Unfortunately, a comprehensive review of the literature on the use of ultrasound imaging to measure and characterize MTrPs didn't fully support the clinical application (Kumbhare et al., 2016). More research is needed to confirm the validity of both magnetic resonance imaging and

ultrasound in detecting MTrPs. Nevertheless, the combination of sEMG and imaging is a promising approach to explore the spatial relationship between the IZ and the MTrP.

A clinical study published in Pain Physician investigated the efficacy of IZ' lidocaine injection for the treatment of MTrPs in patients with chronic neck pain (Xie et al., 2015). The authors acknowledged the motor endplate' dysfunction as the most reasonable explanation for the MTrP' pathophysiology (Kuan, 2009), but quoted details of study on the spatial relationship between the MTrP and the IZ (Barbero et al., 2013) to speculate that two elements may not overlap.

The study design of Xie et al.'s research (2015) was developed test whether any injection site for MTrPs would have a clinical effect on patients' perceptions of the intensity of pain on a visual analogue scale and on their perceptions of painful days per month. Thus, 120 patients with a MTrP in the upper trapezius were randomly divided into 5 experimental groups: (1) injection of saline at the MTrP' location (n = 24), (2) injection of lidocaine at the MTrP' location (n = 24), (3) injection of saline at a mid-upper trapezius IZ' location (n = 24), (4) injection of lidocaine at the mid-upper trapezius IZ' location (n = 24), and (5) combined injection of lidocaine at mid-upper and lower trapezius IZ' locations (n = 24). The study confirmed that a lidocaine injection performed in the IZ' location, when compared with the effects of a MTrP' injection, significantly reduces the intensity and frequency of neck pain at 6 months post-intervention.

The internal validity of the study was fairly good; inclusion/exclusion criteria for patients had been detailed, a random allocation had been adopted and blinding of assessors had been applied. Two weakness in the statistical analysis should be noted, however a sample-size' computation had not been performed and the authors hadn't provided a between-group statistical comparison for the outcomes measures. Overall, both the rationale and the

results of study were interesting and represent possible implications for the IZ within clinical interventions. Notably, the distribution pattern of the IZ in the upper trapezius had been determined during a preliminary time-consuming and complex anatomical study. The authors had examined 44 pieces of trapezius muscle from 22 cadavers and identified the IZ using an intramuscular Sihler's neural staining' technique. Finally, a schematic diagram of the trunk including the distribution pattern of the IZ was obtained to guide IZ' injections. The proposed method to guide an injection into an IZ may be at least questionable, when considering that sEMG can be used to precisely locate the IZ in each patient (Barbero et al., 2011). Similar clinical studies may further explore the efficacy of MTrP-related interventions targeting the IZ by using sEMG. The use of sEMG and an electrodes' array have already been explored for Botulinum toxin injections (Guzman-Venegas et al., 2011). Preliminary results have been promising and have suggested that the proposed methods can be useful in optimising the injection site and to reducing the Botulinum toxin' dosage (Guzman-Venegas et al., 2014, Lapatki et al., 2011).

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Appendix I: List number 1. Epochs' list extracted using customized software. IZ' location estimated by operators A and B is included. Each epoch is identified by a code containing the following details: date of acquisition, subject ID, contraction number, operator code, signal epoch, matrix column. A1, 1st estimate by operator A; A2, 2nd estimate by operator A; B1, 1st estimate by operator B; B2, 2nd estimare by operator B.

n	Epochs_ID	A1	A2	B1	B2
1	20071019_00002_002_mb_00010241_00010752_0001_0011	6	6	6	6
2	20071019_00002_004_rg_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5
3	20071019_00002_005_rg_00010241_00010752_0001_0011	6	5.5	5.5	5.5
4	20071019_00003_002_mb_00010241_00010752_0001_0011	6	6	6	6
5	20071019_00003_005_mb_00010241_00010752_0001_0011	6	6	6	6
6	20071019_00003_006_rg_00010241_00010752_0001_0011	5	5	5	5
7	20071025_00004_002_rg_00010241_00010752_0001_0011	5	5	5	5
8	20071025_00004_003_mb_00010241_00010752_0001_0011	4.5	4.5	4.5	4.5
9	20071025_00004_003_rg_00010241_00010752_0001_0011	5	5	5	5
10	20071025_00004_004_rg_00010241_00010752_0001_0011	5	5	5	5
11	20071025_00004_006_rg_00010241_00010752_0001_0011	5	5	5	5
12	20071026_00005_001_rg_00010241_00010752_0001_0011	4.5	4.5	4.5	4.5
13	20071026_00005_003_mb_00010241_00010752_0001_0011	5	5	5	5
14	20071026_00005_004_mb_00010241_00010752_0001_0011	5	5	5	5
15	20071026_00005_005_mb_00010241_00010752_0001_0011	5.5	5	5	5
16	20071026_00005_005_rg_00010241_00010752_0001_0011	4	4	4	4
17	20071026_00006_002_rg_00010241_00010752_0001_0011	5	5	5	5
18	20071026_00006_004_mb_00010241_00010752_0001_0011	5	5	5	5
19	20071026_00006_006_rg_00010241_00010752_0001_0011	5	5	5	5
20	20071026_00007_001_rg_00010241_00010752_0001_0011	4	4	4	4
21	20071026_00007_002_mb_00010241_00010752_0001_0011	4	4	4	4
22	20071026_00007_003_rg_00010241_00010752_0001_0011	4	3.5	4	4
23	20071026_00007_004_mb_00010241_00010752_0001_0011	4.5	4.5	4.5	4.5
24	20071026_00007_005_mb_00010241_00010752_0001_0011	4.5	4	4	4
25	20071026_00007_006_rg_00010241_00010752_0001_0011	4	4	4	4
26	20071026_00008_001_mb_00010241_00010752_0001_0011	5	5	5	5
27	20071026_00008_002_rg_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5
28	20071026_00008_004_mb_00010241_00010752_0001_0011	5	5	5	5
29	20071026_00008_004_rg_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5
30	20071026_00008_005_rg_00010241_00010752_0001_0011	5	5	5	5
31	20071026_00008_006_mb_00010241_00010752_0001_0011	5	5	5	5
32	20071026_00009_001_rg_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5
33	20071026_00009_002_mb_00010241_00010752_0001_0011	6.5	5.5	6	6
34	20071026_00009_003_mb_00010241_00010752_0001_0011	6.5	6.5	6	6
35	20071026_00009_004_mb_00010241_00010752_0001_0011	6.5	6.5	6.5	6.5
36	20071026_00009_005_rg_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5

37	20071026_00009_006_mb_00010241_00010752_0001_0011	6.5	6.5	7	6.5
38	20071112_00010_002_mb_00010241_00010752_0001_0011	4.5	4.5	4.5	4.5
39	20071112_00011_002_rg_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5
40	20071112_00011_004_mb_00010241_00010752_0001_0011	5.5	5.5	5.5	5.5
41	20071019_00002_002_mb_00010241_00010752_0024_0014	18	18	18	18
42	20071019_00002_004_rg_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
43	20071019_00002_005_rg_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
44	20071019_00003_002_mb_00010241_00010752_0024_0014	18	18	18	18
45	20071019_00003_005_mb_00010241_00010752_0024_0014	18	18	18	18
46	20071019_00003_006_rg_00010241_00010752_0024_0014	19	19	19	19
47	20071025_00004_002_rg_00010241_00010752_0024_0014	18.5	18.5	18	18.5
48	20071025_00004_003_mb_00010241_00010752_0024_0014	19	19	19	19
49	20071025_00004_003_rg_00010241_00010752_0024_0014	18.5	18.5	18	18
50	20071025_00004_004_rg_00010241_00010752_0024_0014	18	18	18	18
51	20071025_00004_006_rg_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
52	20071026_00005_001_rg_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
53	20071026_00005_003_mb_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
54	20071026_00005_004_mb_00010241_00010752_0024_0014	18.5	18.5	19	19
55	20071026_00005_005_mb_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
56	20071026_00005_005_rg_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
57	20071026_00006_002_rg_00010241_00010752_0024_0014	19	19	19	19
58	20071026_00006_004_mb_00010241_00010752_0024_0014	19	19	19	19
59	20071026_00006_006_rg_00010241_00010752_0024_0014	19	19	19	19
60	20071026_00007_001_rg_00010241_00010752_0024_0014	20	19.5	20	19.5
61	20071026_00007_002_mb_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
62	20071026_00007_003_rg_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
63	20071026_00007_004_mb_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
64	20071026_00007_005_mb_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
65	20071026_00007_006_rg_00010241_00010752_0024_0014	19.5	19.5	20	20
66	20071026_00008_001_mb_00010241_00010752_0024_0014	19.5	19.5	19	19
67	20071026_00008_002_rg_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
68	20071026_00008_004_mb_00010241_00010752_0024_0014	19	19	19	19
69	20071026_00008_004_rg_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
70	20071026_00008_005_rg_00010241_00010752_0024_0014	19	19	19	19
71	20071026_00008_006_mb_00010241_00010752_0024_0014	19.5	19	19	19
72	20071026_00009_001_rg_00010241_00010752_0024_0014	19	19	19	19
73	20071026_00009_002_mb_00010241_00010752_0024_0014	18	18	18	18
74	20071026_00009_003_mb_00010241_00010752_0024_0014	18	18	18	18
75	20071026_00009_004_mb_00010241_00010752_0024_0014	18	17.5	18	18
76	20071026_00009_005_rg_00010241_00010752_0024_0014	19	19	19	19
77	20071026_00009_006_mb_00010241_00010752_0024_0014	18	18	18	18
78	20071112_00010_002_mb_00010241_00010752_0024_0014	19.5	19.5	19.5	19.5
79	20071112_00011_002_rg_00010241_00010752_0024_0014	18.5	18.5	19	18.5
80	20071112_00011_004_mb_00010241_00010752_0024_0014	18.5	18.5	18.5	18.5
81	20071019_00002_002_mb_00010241_00010752_0027_0037	31.5	31.5	31.5	31.5
82	20071019_00002_004_rg_00010241_00010752_0027_0037	31	31	31	31
83	20071019_00002_005_rg_00010241_00010752_0027_0037	31	31	33	31

84	20071019_00003_002_mb_00010241_00010752_0027_0037	31.5	31.5	31	31.5
85	20071019_00003_005_mb_00010241_00010752_0027_0037	31	31	31	31
86	20071019_00003_006_rg_00010241_00010752_0027_0037	31	31	31	31
87	20071025_00004_002_rg_00010241_00010752_0027_0037	32	32	32	32
88	20071025_00004_003_mb_00010241_00010752_0027_0037	31.5	31.5	31.5	31.5
89	20071025_00004_003_rg_00010241_00010752_0027_0037	32	32	32	32
90	20071025_00004_004_rg_00010241_00010752_0027_0037	32	32	32	32
91	20071025_00004_006_rg_00010241_00010752_0027_0037	32	32	32	32
92	20071026_00005_001_rg_00010241_00010752_0027_0037	30.5	30.5	30	30
93	20071026_00005_003_mb_00010241_00010752_0027_0037	31.5	31.5	31.5	31.5
94	20071026_00005_004_mb_00010241_00010752_0027_0037	31.5	31.5	31.5	31.5
95	20071026_00005_005_mb_00010241_00010752_0027_0037	31.5	31.5	31.5	31.5
96	20071026_00005_005_rg_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
97	20071026_00006_002_rg_00010241_00010752_0027_0037	31	30.5	30.5	30.5
98	20071026_00006_004_mb_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
99	20071026_00006_006_rg_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
100	20071026_00007_001_rg_00010241_00010752_0027_0037	30	30	30	30
101	20071026_00007_002_mb_00010241_00010752_0027_0037	30.5	31.5	31.5	31.5
102	20071026_00007_003_rg_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
103	20071026_00007_004_mb_00010241_00010752_0027_0037	31	31	30.5	31
104	20071026_00007_005_mb_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
105	20071026_00007_006_rg_00010241_00010752_0027_0037	30.5	30.5	30	30
106	20071026_00008_001_mb_00010241_00010752_0027_0037	30	30.5	30	30
107	20071026_00008_002_rg_00010241_00010752_0027_0037	31	31	31	31
108	20071026_00008_004_mb_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
109	20071026_00008_004_rg_00010241_00010752_0027_0037	31	31	31	31
110	20071026_00008_005_rg_00010241_00010752_0027_0037	31	31	31	31
111	20071026_00008_006_mb_00010241_00010752_0027_0037	30	30	30	30
112	20071026_00009_001_rg_00010241_00010752_0027_0037	30.5	30.5	31	31
113	20071026_00009_002_mb_00010241_00010752_0027_0037	32	32	32	32
114	20071026_00009_003_mb_00010241_00010752_0027_0037	32	32	32	32
115	20071026_00009_004_mb_00010241_00010752_0027_0037	32	32	32	32
116	20071026_00009_005_rg_00010241_00010752_0027_0037	31	31	31	31
117	20071026_00009_006_mb_00010241_00010752_0027_0037	31.5	32	31.5	31.5
118	20071112_00010_002_mb_00010241_00010752_0027_0037	30.5	30.5	30.5	30.5
119	20071112_00011_002_rg_00010241_00010752_0027_0037	31	31	31	31
120	20071112_00011_004_mb_00010241_00010752_0027_0037	31	31	31	31
121	20071019_00002_002_mb_00010241_00010752_0050_0040	45	45	45	45
122	20071019_00002_004_rg_00010241_00010752_0050_0040	45	45	45	45
123	20071019_00002_005_rg_00010241_00010752_0050_0040	45	45	45	45
124	20071019_00003_002_mb_00010241_00010752_0050_0040	45.5	45.5	46	46
125	20071019_00003_005_mb_00010241_00010752_0050_0040	45.5	45.5	46	45.5
126	20071019_00003_006_rg_00010241_00010752_0050_0040	45	45	45	45
127	20071025_00004_002_rg_00010241_00010752_0050_0040	44	44	44	44
128	20071025_00004_003_mb_00010241_00010752_0050_0040	44	44	44	44
129	20071025_00004_003_rg_00010241_00010752_0050_0040	44	44	44	44
130	20071025_00004_004_rg_00010241_00010752_0050_0040	44	44	44	44

131	20071025_00004_006_rg_00010241_00010752_0050_0040	44	44	44	44
132	20071026_00005_001_rg_00010241_00010752_0050_0040	46	46	46	46
133	20071026_00005_003_mb_00010241_00010752_0050_0040	45	45	45	45
134	20071026_00005_004_mb_00010241_00010752_0050_0040	44.5	44.5	44.5	44.5
135	20071026_00005_005_mb_00010241_00010752_0050_0040	45	45	45	45
136	20071026_00005_005_rg_00010241_00010752_0050_0040	46	46	46	46
137	20071026_00006_002_rg_00010241_00010752_0050_0040	46	46	46	46
138	20071026_00006_004_mb_00010241_00010752_0050_0040	45.5	45.5	46	46
139	20071026_00006_006_rg_00010241_00010752_0050_0040	45.5	45.5	46	45.5
140	20071026_00007_001_rg_00010241_00010752_0050_0040	46	46	46	46
141	20071026_00007_002_mb_00010241_00010752_0050_0040	46	46	46	46
142	20071026_00007_003_rg_00010241_00010752_0050_0040	46	46	46	46
143	20071026_00007_004_mb_00010241_00010752_0050_0040	45.5	45.5	45.5	46
144	20071026_00007_005_mb_00010241_00010752_0050_0040	45.5	46	45.5	45.5
145	20071026_00007_006_rg_00010241_00010752_0050_0040	46	46	46	46
146	20071026_00008_001_mb_00010241_00010752_0050_0040	46	46	46	46
147	20071026_00008_002_rg_00010241_00010752_0050_0040	45	45	45	45
148	20071026_00008_004_mb_00010241_00010752_0050_0040	46	45.5	46	46
149	20071026_00008_004_rg_00010241_00010752_0050_0040	45	45	45	45
150	20071026_00008_005_rg_00010241_00010752_0050_0040	45	45	45	45
151	20071026_00008_006_mb_00010241_00010752_0050_0040	46	46	46	46
152	20071026_00009_001_rg_00010241_00010752_0050_0040	46	46	46	46
153	20071026_00009_002_mb_00010241_00010752_0050_0040	44.5	44.5	44.5	45
154	20071026_00009_003_mb_00010241_00010752_0050_0040	44.5	44.5	44.5	44.5
155	20071026_00009_004_mb_00010241_00010752_0050_0040	44.5	44.5	44.5	44.5
156	20071026_00009_005_rg_00010241_00010752_0050_0040	46	46	46	46
157	20071026_00009_006_mb_00010241_00010752_0050_0040	44.5	44.5	44.5	44.5
158	20071112_00010_002_mb_00010241_00010752_0050_0040	47	47	47	47
159	20071112_00011_002_rg_00010241_00010752_0050_0040	45.5	45.5	45.5	45.5
160	20071112_00011_004_mb_00010241_00010752_0050_0040	45.5	45.5	45.5	45.5
161	20071019_00002_002_mb_00010241_00010752_0053_0063	57	57	57	57
162	20071019_00002_004_rg_00010241_00010752_0053_0063	55.5	55.5	56	56
163	20071019_00002_005_rg_00010241_00010752_0053_0063	56	56	56	56
164	20071019_00003_002_mb_00010241_00010752_0053_0063	56	56	56	56
165	20071019_00003_005_mb_00010241_00010752_0053_0063	56	56	56	56
166	20071019_00003_006_rg_00010241_00010752_0053_0063	56	56	56	56
167	20071025_00004_002_rg_00010241_00010752_0053_0063	58	58	58	58
168	20071025_00004_003_mb_00010241_00010752_0053_0063	57.5	57.5	58	58
169	20071025_00004_003_rg_00010241_00010752_0053_0063	58	58	58	58
170	20071025_00004_004_rg_00010241_00010752_0053_0063	58	58	58	58
171	20071025_00004_006_rg_00010241_00010752_0053_0063	57.5	57.5	58	58
172	20071026_00005_001_rg_00010241_00010752_0053_0063	55.5	56	56	56
173	20071026_00005_003_mb_00010241_00010752_0053_0063	57	57	57	56.5
174	20071026_00005_004_mb_00010241_00010752_0053_0063	57	57	57	57
175	20071026_00005_005_mb_00010241_00010752_0053_0063	57	57	57	57
176	20071026_00005_005_rg_00010241_00010752_0053_0063	55.5	55.5	56	55.5
177	20071026_00006_002_rg_00010241_00010752_0053_0063	56	56	56	56

178	20071026_00006_004_mb_00010241_00010752_0053_0063	56	56	56	56
179	20071026_00006_006_rg_00010241_00010752_0053_0063	56	56	56	56
180	20071026_00007_001_rg_00010241_00010752_0053_0063	56	56.5	56	56
181	20071026_00007_002_mb_00010241_00010752_0053_0063	56	56	56	56
182	20071026_00007_003_rg_00010241_00010752_0053_0063	56	56	56	56
183	20071026_00007_004_mb_00010241_00010752_0053_0063	56	56	56	56
184	20071026_00007_005_mb_00010241_00010752_0053_0063	56.5	56	56.5	56.5
185	20071026_00007_006_rg_00010241_00010752_0053_0063	56	56	56	56
186	20071026_00008_001_mb_00010241_00010752_0053_0063	56	56	56	56
187	20071026_00008_002_rg_00010241_00010752_0053_0063	56.5	56.5	56.5	56.5
188	20071026_00008_004_mb_00010241_00010752_0053_0063	56	56	56	56
189	20071026_00008_004_rg_00010241_00010752_0053_0063	56.5	56.5	56.5	56
190	20071026_00008_005_rg_00010241_00010752_0053_0063	56.5	56.5	56.5	56.5
191	20071026_00008_006_mb_00010241_00010752_0053_0063	56	56	56	56
192	20071026_00009_001_rg_00010241_00010752_0053_0063	56	56	56	56
193	20071026_00009_002_mb_00010241_00010752_0053_0063	57	57	57	57
194	20071026_00009_003_mb_00010241_00010752_0053_0063	57	57	57	57
195	20071026_00009_004_mb_00010241_00010752_0053_0063	57	57	57	57
196	20071026_00009_005_rg_00010241_00010752_0053_0063	56	56	56	56
197	20071026_00009_006_mb_00010241_00010752_0053_0063	57	57	57	57
198	20071112_00010_002_mb_00010241_00010752_0053_0063	55	55	55	55
199	20071112_00011_002_rg_00010241_00010752_0053_0063	56	56	56	56
200	20071112_00011_004_mb_00010241_00010752_0053_0063	56	56	56	56

Appendix II: List number 2. Epochs' list extracted using customized software. IZ' location estimated by operators A is included. Each epoch is identified by a code containing the following details: date of acquisition, subject ID, contraction number, operator code, signal epoch, matrix column. A1, 1st estimate by operator A.

n	Epochs_ID	A1
1	20071019_00002_001_mb_00010241_00010752_0001_0011	6
2	20071019_00002_003_mb_00010241_00010752_0001_0011	6
3	20071019_00003_002_mb_00010241_00010752_0001_0011	6
4	20071019_00003_006_mb_00010241_00010752_0001_0011	6
5	20071025_00004_003_mb_00010241_00010752_0001_0011	4.5
6	20071025_00004_006_mb_00010241_00010752_0001_0011	4.5
7	20071026_00005_001_mb_00010241_00010752_0001_0011	5
8	20071026_00005_003_mb_00010241_00010752_0001_0011	5
9	20071026_00006_001_mb_00010241_00010752_0001_0011	5
10	20071026_00006_005_mb_00010241_00010752_0001_0011	5
11	20071026_00007_003_mb_00010241_00010752_0001_0011	4
12	20071026_00007_006_mb_00010241_00010752_0001_0011	4
13	20071026_00008_004_mb_00010241_00010752_0001_0011	5
14	20071026_00008_006_mb_00010241_00010752_0001_0011	5
15	20071026_00009_001_mb_00010241_00010752_0001_0011	6
16	20071026_00009_002_mb_00010241_00010752_0001_0011	6
17	20071112_00010_001_mb_00010241_00010752_0001_0011	4.5
18	20071112_00010_005_mb_00010241_00010752_0001_0011	4.5
19	20071112_00011_002_mb_00010241_00010752_0001_0011	5.5
20	20071112_00011_005_mb_00010241_00010752_0001_0011	5.5
21	20071019_00002_001_mb_00010241_00010752_0024_0014	18
22	20071019_00002_003_mb_00010241_00010752_0024_0014	18
23	20071019_00003_002_mb_00010241_00010752_0024_0014	18
24	20071019_00003_006_mb_00010241_00010752_0024_0014	18
25	20071025_00004_003_mb_00010241_00010752_0024_0014	19
26	20071025_00004_006_mb_00010241_00010752_0024_0014	19
27	20071026_00005_001_mb_00010241_00010752_0024_0014	18.5
28	20071026_00005_003_mb_00010241_00010752_0024_0014	18.5
29	20071026_00006_001_mb_00010241_00010752_0024_0014	19
30	20071026_00006_005_mb_00010241_00010752_0024_0014	19
31	20071026_00007_003_mb_00010241_00010752_0024_0014	20
32	20071026_00007_006_mb_00010241_00010752_0024_0014	19
33	20071026_00008_004_mb_00010241_00010752_0024_0014	19
34	20071026_00008_006_mb_00010241_00010752_0024_0014	19
35	20071026_00009_001_mb_00010241_00010752_0024_0014	18
36	20071026_00009_002_mb_00010241_00010752_0024_0014	18
37	20071112_00010_001_mb_00010241_00010752_0024_0014	19.5

38	20071112_00010_005_mb_00010241_00010752_0024_0014	19.5
39	20071112_00011_002_mb_00010241_00010752_0024_0014	19
40	20071112_00011_005_mb_00010241_00010752_0024_0014	18.5
41	20071019_00002_001_mb_00010241_00010752_0027_0037	31.5
42	20071019_00002_003_mb_00010241_00010752_0027_0037	31.5
43	20071019_00003_002_mb_00010241_00010752_0027_0037	31
44	20071019_00003_006_mb_00010241_00010752_0027_0037	31
45	20071025_00004_003_mb_00010241_00010752_0027_0037	31.5
46	20071025_00004_006_mb_00010241_00010752_0027_0037	31.5
47	20071026_00005_001_mb_00010241_00010752_0027_0037	31.5
48	20071026_00005_003_mb_00010241_00010752_0027_0037	31.5
49	20071026_00006_001_mb_00010241_00010752_0027_0037	30.5
50	20071026_00006_005_mb_00010241_00010752_0027_0037	30.5
51	20071026_00007_003_mb_00010241_00010752_0027_0037	30
52	20071026_00007_006_mb_00010241_00010752_0027_0037	30.5
53	20071026_00008_004_mb_00010241_00010752_0027_0037	30.5
54	20071026_00008_006_mb_00010241_00010752_0027_0037	30
55	20071026_00009_001_mb_00010241_00010752_0027_0037	32
56	20071026_00009_002_mb_00010241_00010752_0027_0037	32
57	20071112_00010_001_mb_00010241_00010752_0027_0037	30
58	20071112_00010_005_mb_00010241_00010752_0027_0037	30
59	20071112_00011_002_mb_00010241_00010752_0027_0037	31
60	20071112_00011_005_mb_00010241_00010752_0027_0037	31.5
61	20071019_00002_001_mb_00010241_00010752_0050_0040	45
62	20071019_00002_003_mb_00010241_00010752_0050_0040	45
63	20071019_00003_002_mb_00010241_00010752_0050_0040	46
64	20071019_00003_006_mb_00010241_00010752_0050_0040	45
65	20071025_00004_003_mb_00010241_00010752_0050_0040	44
66	20071025_00004_006_mb_00010241_00010752_0050_0040	44
67	20071026_00005_001_mb_00010241_00010752_0050_0040	45
68	20071026_00005_003_mb_00010241_00010752_0050_0040	45
69	20071026_00006_001_mb_00010241_00010752_0050_0040	45.5
70	20071026_00006_005_mb_00010241_00010752_0050_0040	46
71	20071026_00007_003_mb_00010241_00010752_0050_0040	46
72	20071026_00007_006_mb_00010241_00010752_0050_0040	46
73	20071026_00008_004_mb_00010241_00010752_0050_0040	46
74	20071026_00008_006_mb_00010241_00010752_0050_0040	46
75	20071026_00009_001_mb_00010241_00010752_0050_0040	45
76	20071026_00009_002_mb_00010241_00010752_0050_0040	44.5
77	20071112_00010_001_mb_00010241_00010752_0050_0040	46.5
78	20071112_00010_005_mb_00010241_00010752_0050_0040	47
79	20071112_00011_002_mb_00010241_00010752_0050_0040	45.5
80	20071112_00011_005_mb_00010241_00010752_0050_0040	45
81	20071019_00002_001_mb_00010241_00010752_0053_0063	57
82	20071019_00002_003_mb_00010241_00010752_0053_0063	57
83	20071019_00003_002_mb_00010241_00010752_0053_0063	56
84	20071019_00003_006_mb_00010241_00010752_0053_0063	56

85	20071025_00004_003_mb_00010241_00010752_0053_0063	58
86	20071025_00004_006_mb_00010241_00010752_0053_0063	58
87	20071026_00005_001_mb_00010241_00010752_0053_0063	57
88	20071026_00005_003_mb_00010241_00010752_0053_0063	57
89	20071026_00006_001_mb_00010241_00010752_0053_0063	56
90	20071026_00006_005_mb_00010241_00010752_0053_0063	56
91	20071026_00007_003_mb_00010241_00010752_0053_0063	56
92	20071026_00007_006_mb_00010241_00010752_0053_0063	56
93	20071026_00008_004_mb_00010241_00010752_0053_0063	56
94	20071026_00008_006_mb_00010241_00010752_0053_0063	56
95	20071026_00009_001_mb_00010241_00010752_0053_0063	57
96	20071026_00009_002_mb_00010241_00010752_0053_0063	57
97	20071112_00010_001_mb_00010241_00010752_0053_0063	55
98	20071112_00010_005_mb_00010241_00010752_0053_0063	54.5
99	20071112_00011_002_mb_00010241_00010752_0053_0063	56
100	20071112_00011_005_mb_00010241_00010752_0053_0063	56.5

Appendix III: List number 3. Epochs' list extracted using customized software. IZ' location estimated by operators A is included. Each epoch is identified by a code containing the following details: date of acquisition. subject ID, contraction number, operator code, signal epoch, matrix column. A1, 1st estimate by operator A.

n	Epochs_ID	A1
1	20071019_00002_005_rg_00000001_00000512_0001_0011	5.5
2	20071019_00002_005_rg_00000513_00001024_0001_0011	5.5
3	20071019_00002_005_rg_00010753_00011264_0001_0011	6
4	20071019_00002_005_rg_00015361_00015872_0001_0011	5.5
5	20071019_00003_003_rg_00004609_00005120_0001_0011	5
6	20071019_00003_003_rg_00007681_00008192_0001_0011	5
7	20071019_00003_003_rg_00015873_00016384_0001_0011	5
8	20071019_00003_003_rg_00019969_00020480_0001_0011	5
9	20071019_00003_006_mb_00003073_00003584_0001_0011	6
10	20071019_00003_006_mb_00005121_00005632_0001_0011	6
11	20071019_00003_006_mb_00007681_00008192_0001_0011	6
12	20071019_00003_006_mb_00011265_00011776_0001_0011	6
13	20071025_00004_005_mb_00007681_00008192_0001_0011	4.5
14	20071025_00004_005_mb_00009217_00009728_0001_0011	4.5
15	20071025_00004_005_mb_00010753_00011264_0001_0011	4.5
16	20071025_00004_005_mb_00011777_00012288_0001_0011	4.5
17	20071025_00004_006_rg_00003585_00004096_0001_0011	5
18	20071025_00004_006_rg_00008193_00008704_0001_0011	5
19	20071025_00004_006_rg_00016385_00016896_0001_0011	5
20	20071025_00004_006_rg_00017409_00017920_0001_0011	5.5
21	20071026_00006_002_rg_00000513_00001024_0001_0011	5
22	20071026_00006_002_rg_00006145_00006656_0001_0011	5
23	20071026_00006_002_rg_00010241_00010752_0001_0011	5
24	20071026_00006_002_rg_00018433_00018944_0001_0011	5
25	20071026_00007_004_mb_00001025_00001536_0001_0011	4
26	20071026_00007_004_mb_00001537_00002048_0001_0011	4
27	20071026_00007_004_mb_00002049_00002560_0001_0011	4
28	20071026_00007_004_mb_00006145_00006656_0001_0011	4
29	20071026_00007_006_mb_00001025_00001536_0001_0011	4
30	20071026_00007_006_mb_00003585_00004096_0001_0011	4
31	20071026_00007_006_mb_00005633_00006144_0001_0011	4
32	20071026_00007_006_mb_00009217_00009728_0001_0011	4
33	20071026_00009_005_rg_00009217_00009728_0001_0011	5.5
34	20071026_00009_005_rg_00009729_00010240_0001_0011	5.5
35	20071026_00009_005_rg_00018945_00019456_0001_0011	5.5
36	20071026_00009_005_rg_00019969_00020480_0001_0011	5.5
37	20071112_00011_004_rg_00007681_00008192_0001_0011	5.5
38	20071112_00011_004_rg_00014337_00014848_0001_0011	5.5

39	20071112_00011_004_rg_00016897_00017408_0001_0011	5.5
40	20071112_00011_004_rg_00017921_00018432_0001_0011	5.5
41	20071019_00002_005_rg_00000001_00000512_0024_0014	18.5
42	20071019_00002_005_rg_00000513_00001024_0024_0014	18.5
43	20071019_00002_005_rg_00010753_00011264_0024_0014	18.5
44	20071019_00002_005_rg_00015361_00015872_0024_0014	18.5
45	20071019_00003_003_rg_00004609_00005120_0024_0014	19
46	20071019_00003_003_rg_00007681_00008192_0024_0014	19
47	20071019_00003_003_rg_00015873_00016384_0024_0014	19
48	20071019_00003_003_rg_00019969_00020480_0024_0014	19
49	20071019_00003_006_mb_00003073_00003584_0024_0014	18
50	20071019_00003_006_mb_00005121_00005632_0024_0014	18
51	20071019_00003_006_mb_00007681_00008192_0024_0014	18
52	20071019_00003_006_mb_00011265_00011776_0024_0014	18
53	20071025_00004_005_mb_00007681_00008192_0024_0014	19
54	20071025_00004_005_mb_00009217_00009728_0024_0014	19
55	20071025_00004_005_mb_00010753_00011264_0024_0014	19
56	20071025_00004_005_mb_00011777_00012288_0024_0014	19
57	20071025_00004_006_rg_00003585_00004096_0024_0014	18.5
58	20071025_00004_006_rg_00008193_00008704_0024_0014	18.5
59	20071025_00004_006_rg_00016385_00016896_0024_0014	18.5
60	20071025_00004_006_rg_00017409_00017920_0024_0014	18.5
61	20071026_00006_002_rg_00000513_00001024_0024_0014	19
62	20071026_00006_002_rg_00006145_00006656_0024_0014	19
63	20071026_00006_002_rg_00010241_00010752_0024_0014	19
64	20071026_00006_002_rg_00018433_00018944_0024_0014	19
65	20071026_00007_004_mb_00001025_00001536_0024_0014	19
66	20071026_00007_004_mb_00001537_00002048_0024_0014	19.5
67	20071026_00007_004_mb_00002049_00002560_0024_0014	19
68	20071026_00007_004_mb_00006145_00006656_0024_0014	19
69	20071026_00007_006_mb_00001025_00001536_0024_0014	19
70	20071026_00007_006_mb_00003585_00004096_0024_0014	19.5
71	20071026_00007_006_mb_00005633_00006144_0024_0014	19.5
72	20071026_00007_006_mb_00009217_00009728_0024_0014	19.5
73	20071026_00009_005_rg_00009217_00009728_0024_0014	19
74	20071026_00009_005_rg_00009729_00010240_0024_0014	19
75	20071026_00009_005_rg_00018945_00019456_0024_0014	19
76	20071026_00009_005_rg_00019969_00020480_0024_0014	19
77	20071112_00011_004_rg_00007681_00008192_0024_0014	19
78	20071112_00011_004_rg_00014337_00014848_0024_0014	19
79	20071112_00011_004_rg_00016897_00017408_0024_0014	19
80	20071112_00011_004_rg_00017921_00018432_0024_0014	19
81	20071019_00002_005_rg_00000001_00000512_0027_0037	31
82	20071019_00002_005_rg_00000513_00001024_0027_0037	31
83	20071019_00002_005_rg_00010753_00011264_0027_0037	31
84	20071019_00002_005_rg_00015361_00015872_0027_0037	31
85	20071019_00003_003_rg_00004609_00005120_0027_0037	31

86	20071019_00003_003_rg_00007681_00008192_0027_0037	31
87	20071019_00003_003_rg_00015873_00016384_0027_0037	31
88	20071019_00003_003_rg_00019969_00020480_0027_0037	31
89	20071019_00003_006_mb_00003073_00003584_0027_0037	31
90	20071019_00003_006_mb_00005121_00005632_0027_0037	31.5
91	20071019_00003_006_mb_00007681_00008192_0027_0037	31
92	20071019_00003_006_mb_00011265_00011776_0027_0037	31.5
93	20071025_00004_005_mb_00007681_00008192_0027_0037	31.5
94	20071025_00004_005_mb_00009217_00009728_0027_0037	31.5
95	20071025_00004_005_mb_00010753_00011264_0027_0037	31.5
96	20071025_00004_005_mb_00011777_00012288_0027_0037	31.5
97	20071025_00004_006_rg_00003585_00004096_0027_0037	32
98	20071025_00004_006_rg_00008193_00008704_0027_0037	32
99	20071025_00004_006_rg_00016385_00016896_0027_0037	32
100	20071025_00004_006_rg_00017409_00017920_0027_0037	32
101	20071026_00006_002_rg_00000513_00001024_0027_0037	30.5
102	20071026_00006_002_rg_00006145_00006656_0027_0037	31
103	20071026_00006_002_rg_00010241_00010752_0027_0037	30.5
104	20071026_00006_002_rg_00018433_00018944_0027_0037	31
105	20071026_00007_004_mb_00001025_00001536_0027_0037	30.5
106	20071026_00007_004_mb_00001537_00002048_0027_0037	30.5
107	20071026_00007_004_mb_00002049_00002560_0027_0037	31
108	20071026_00007_004_mb_00006145_00006656_0027_0037	30.5
109	20071026_00007_006_mb_00001025_00001536_0027_0037	30.5
110	20071026_00007_006_mb_00003585_00004096_0027_0037	30.5
111	20071026_00007_006_mb_00005633_00006144_0027_0037	30.5
112	20071026_00007_006_mb_00009217_00009728_0027_0037	30.5
113	20071026_00009_005_rg_00009217_00009728_0027_0037	31
114	20071026_00009_005_rg_00009729_00010240_0027_0037	30.5
115	20071026_00009_005_rg_00018945_00019456_0027_0037	31
116	20071026_00009_005_rg_00019969_00020480_0027_0037	30.5
117	20071112_00011_004_rg_00007681_00008192_0027_0037	31
118	20071112_00011_004_rg_00014337_00014848_0027_0037	31
119	20071112_00011_004_rg_00016897_00017408_0027_0037	31
120	20071112_00011_004_rg_00017921_00018432_0027_0037	31
121	20071019_00002_005_rg_00000001_00000512_0050_0040	45
122	20071019_00002_005_rg_00000513_00001024_0050_0040	45
123	20071019_00002_005_rg_00010753_00011264_0050_0040	45.5
124	20071019_00002_005_rg_00015361_00015872_0050_0040	45
125	20071019_00003_003_rg_00004609_00005120_0050_0040	45.5
126	20071019_00003_003_rg_00007681_00008192_0050_0040	46
127	20071019_00003_003_rg_00015873_00016384_0050_0040	45
128	20071019_00003_003_rg_00019969_00020480_0050_0040	45.5
129	20071019_00003_006_mb_00003073_00003584_0050_0040	45
130	20071019_00003_006_mb_00005121_00005632_0050_0040	45
131	20071019_00003_006_mb_00007681_00008192_0050_0040	45
132	20071019_00003_006_mb_00011265_00011776_0050_0040	45.5

133	20071025_00004_005_mb_00007681_00008192_0050_0040	44
134	20071025_00004_005_mb_00009217_00009728_0050_0040	44
135	20071025_00004_005_mb_00010753_00011264_0050_0040	44
136	20071025_00004_005_mb_00011777_00012288_0050_0040	44
137	20071025_00004_006_rg_00003585_00004096_0050_0040	44
138	20071025_00004_006_rg_00008193_00008704_0050_0040	44
139	20071025_00004_006_rg_00016385_00016896_0050_0040	44
140	20071025_00004_006_rg_00017409_00017920_0050_0040	44
141	20071026_00006_002_rg_00000513_00001024_0050_0040	46
142	20071026_00006_002_rg_00006145_00006656_0050_0040	46
143	20071026_00006_002_rg_00010241_00010752_0050_0040	46
144	20071026_00006_002_rg_00018433_00018944_0050_0040	46
145	20071026_00007_004_mb_00001025_00001536_0050_0040	45.5
146	20071026_00007_004_mb_00001537_00002048_0050_0040	45.5
147	20071026_00007_004_mb_00002049_00002560_0050_0040	45.5
148	20071026_00007_004_mb_00006145_00006656_0050_0040	46
149	20071026_00007_006_mb_00001025_00001536_0050_0040	45.5
150	20071026_00007_006_mb_00003585_00004096_0050_0040	45.5
151	20071026_00007_006_mb_00005633_00006144_0050_0040	46
152	20071026_00007_006_mb_00009217_00009728_0050_0040	46
153	20071026_00009_005_rg_00009217_00009728_0050_0040	46
154	20071026_00009_005_rg_00009729_00010240_0050_0040	46
155	20071026_00009_005_rg_00018945_00019456_0050_0040	46
156	20071026_00009_005_rg_00019969_00020480_0050_0040	46
157	20071112_00011_004_rg_00007681_00008192_0050_0040	46
158	20071112_00011_004_rg_00014337_00014848_0050_0040	45.5
159	20071112_00011_004_rg_00016897_00017408_0050_0040	46
160	20071112_00011_004_rg_00017921_00018432_0050_0040	45.5
161	20071019_00002_005_rg_00000001_00000512_0053_0063	56
162	20071019_00002_005_rg_00000513_00001024_0053_0063	56
163	20071019_00002_005_rg_00010753_00011264_0053_0063	55.5
164	20071019_00002_005_rg_00015361_00015872_0053_0063	56
165	20071019_00003_003_rg_00004609_00005120_0053_0063	56
166	20071019_00003_003_rg_00007681_00008192_0053_0063	56
167	20071019_00003_003_rg_00015873_00016384_0053_0063	56.5
168	20071019_00003_003_rg_00019969_00020480_0053_0063	56
169	20071019_00003_006_mb_00003073_00003584_0053_0063	56
170	20071019_00003_006_mb_00005121_00005632_0053_0063	56
171	20071019_00003_006_mb_00007681_00008192_0053_0063	56
172	20071019_00003_006_mb_00011265_00011776_0053_0063	56
173	20071025_00004_005_mb_00007681_00008192_0053_0063	57.5
174	20071025_00004_005_mb_00009217_00009728_0053_0063	57.5
175	20071025_00004_005_mb_00010753_00011264_0053_0063	57.5
176	20071025_00004_005_mb_00011777_00012288_0053_0063	57.5
177	20071025_00004_006_rg_00003585_00004096_0053_0063	57.5
178	20071025_00004_006_rg_00008193_00008704_0053_0063	58
179	20071025_00004_006_rg_00016385_00016896_0053_0063	58

180	20071025_00004_006_rg_00017409_00017920_0053_0063	58
181	20071026_00006_002_rg_00000513_00001024_0053_0063	56
182	20071026_00006_002_rg_00006145_00006656_0053_0063	56
183	20071026_00006_002_rg_00010241_00010752_0053_0063	56
184	20071026_00006_002_rg_00018433_00018944_0053_0063	56
185	20071026_00007_004_mb_00001025_00001536_0053_0063	56
186	20071026_00007_004_mb_00001537_00002048_0053_0063	56
187	20071026_00007_004_mb_00002049_00002560_0053_0063	56
188	20071026_00007_004_mb_00006145_00006656_0053_0063	56
189	20071026_00007_006_mb_00001025_00001536_0053_0063	56
190	20071026_00007_006_mb_00003585_00004096_0053_0063	56
191	20071026_00007_006_mb_00005633_00006144_0053_0063	56
192	20071026_00007_006_mb_00009217_00009728_0053_0063	56
193	20071026_00009_005_rg_00009217_00009728_0053_0063	56
194	20071026_00009_005_rg_00009729_00010240_0053_0063	56
195	20071026_00009_005_rg_00018945_00019456_0053_0063	56
196	20071026_00009_005_rg_00019969_00020480_0053_0063	56
197	20071112_00011_004_rg_00007681_00008192_0053_0063	56
198	20071112_00011_004_rg_00014337_00014848_0053_0063	56
199	20071112_00011_004_rg_00016897_00017408_0053_0063	56
200	20071112_00011_004_rg_00017921_00018432_0053_0063	56

Appendix IV: List number 4. Epochs' list extracted using customized software. IZ' location estimated by operators A is included. Each epoch is identified by a code containing the following details: date of acquisition. subject ID. contraction number, operator code, signal epoch, matrix column. A1, 1st estimate by operator A.

n	Epochs_ID	A
1	20071019_00002_001_rg_00010241_00010752_0001_0011	5.5
2	20071019_00002_002_rg_00010241_00010752_0001_0011	5.5
3	20071019_00002_003_rg_00010241_00010752_0001_0011	6
4	20071019_00002_004_rg_00010241_00010752_0001_0011	5.5
5	20071019_00002_005_rg_00010241_00010752_0001_0011	5.5
6	20071019_00002_006_rg_00010241_00010752_0001_0011	5.5
7	20071019_00003_001_rg_00010241_00010752_0001_0011	5
8	20071019_00003_002_rg_00010241_00010752_0001_0011	5
9	20071019_00003_003_rg_00010241_00010752_0001_0011	5
10	20071019_00003_004_rg_00010241_00010752_0001_0011	5
11	20071019_00003_005_rg_00010241_00010752_0001_0011	5
12	20071019_00003_006_rg_00010241_00010752_0001_0011	5
13	20071025_00004_001_rg_00010241_00010752_0001_0011	5
14	20071025_00004_002_rg_00010241_00010752_0001_0011	5
15	20071025_00004_003_rg_00010241_00010752_0001_0011	5
16	20071025_00004_004_rg_00010241_00010752_0001_0011	5
17	20071025_00004_005_rg_00010241_00010752_0001_0011	5
18	20071025_00004_006_rg_00010241_00010752_0001_0011	5
19	20071026_00005_001_rg_00010241_00010752_0001_0011	4.5
20	20071026_00005_002_rg_00010241_00010752_0001_0011	4
21	20071026_00005_003_rg_00010241_00010752_0001_0011	4
22	20071026_00005_004_rg_00010241_00010752_0001_0011	4
23	20071026_00005_005_rg_00010241_00010752_0001_0011	4
24	20071026_00005_006_rg_00010241_00010752_0001_0011	4
25	20071026_00006_001_rg_00010241_00010752_0001_0011	5
26	20071026_00006_002_rg_00010241_00010752_0001_0011	5
27	20071026_00006_003_rg_00010241_00010752_0001_0011	5
28	20071026_00006_004_rg_00010241_00010752_0001_0011	5
29	20071026_00006_005_rg_00010241_00010752_0001_0011	5
30	20071026_00006_006_rg_00010241_00010752_0001_0011	5
31	20071026_00007_001_rg_00010241_00010752_0001_0011	4
32	20071026_00007_002_rg_00010241_00010752_0001_0011	4
33	20071026_00007_003_rg_00010241_00010752_0001_0011	4
34	20071026_00007_004_rg_00010241_00010752_0001_0011	4
35	20071026_00007_005_rg_00010241_00010752_0001_0011	4
36	20071026_00007_006_rg_00010241_00010752_0001_0011	4
37	20071026_00008_001_rg_00010241_00010752_0001_0011	5.5
38	20071026_00008_002_rg_00010241_00010752_0001_0011	5.5

39	20071026_00008_003_rg_00010241_00010752_0001_0011	6
40	20071026_00008_004_rg_00010241_00010752_0001_0011	5.5
41	20071026_00008_005_rg_00010241_00010752_0001_0011	5
42	20071026_00008_006_rg_00010241_00010752_0001_0011	5.5
43	20071026_00009_001_rg_00010241_00010752_0001_0011	5.5
44	20071026_00009_002_rg_00010241_00010752_0001_0011	5.5
45	20071026_00009_003_rg_00010241_00010752_0001_0011	5.5
46	20071026_00009_004_rg_00010241_00010752_0001_0011	5.5
47	20071026_00009_005_rg_00010241_00010752_0001_0011	5.5
48	20071026_00009_006_rg_00010241_00010752_0001_0011	5.5
49	20071112_00010_001_rg_00010241_00010752_0001_0011	4
50	20071112_00010_002_rg_00010241_00010752_0001_0011	4
51	20071112_00010_003_rg_00010241_00010752_0001_0011	4.5
52	20071112_00010_004_rg_00010241_00010752_0001_0011	4.5
53	20071112_00010_005_rg_00010241_00010752_0001_0011	4.5
54	20071112_00010_006_rg_00010241_00010752_0001_0011	4.5
55	20071112_00011_001_rg_00010241_00010752_0001_0011	6
56	20071112_00011_002_rg_00010241_00010752_0001_0011	5.5
57	20071112_00011_003_rg_00010241_00010752_0001_0011	5
58	20071112_00011_004_rg_00010241_00010752_0001_0011	5
59	20071112_00011_005_rg_00010241_00010752_0001_0011	5.5
60	20071112_00011_006_rg_00010241_00010752_0001_0011	5
61	20071019_00002_001_rg_00010241_00010752_0024_0014	18.5
62	20071019_00002_002_rg_00010241_00010752_0024_0014	18.5
63	20071019_00002_003_rg_00010241_00010752_0024_0014	18.5
64	20071019_00002_004_rg_00010241_00010752_0024_0014	18.5
65	20071019_00002_005_rg_00010241_00010752_0024_0014	18.5
66	20071019_00002_006_rg_00010241_00010752_0024_0014	18.5
67	20071019_00003_001_rg_00010241_00010752_0024_0014	19
68	20071019_00003_002_rg_00010241_00010752_0024_0014	18.5
69	20071019_00003_003_rg_00010241_00010752_0024_0014	19
70	20071019_00003_004_rg_00010241_00010752_0024_0014	19
71	20071019_00003_005_rg_00010241_00010752_0024_0014	19
72	20071019_00003_006_rg_00010241_00010752_0024_0014	19
73	20071025_00004_001_rg_00010241_00010752_0024_0014	18
74	20071025_00004_002_rg_00010241_00010752_0024_0014	18
75	20071025_00004_003_rg_00010241_00010752_0024_0014	18
76	20071025_00004_004_rg_00010241_00010752_0024_0014	18
77	20071025_00004_005_rg_00010241_00010752_0024_0014	18
78	20071025_00004_006_rg_00010241_00010752_0024_0014	18.5
79	20071026_00005_001_rg_00010241_00010752_0024_0014	19.5
80	20071026_00005_002_rg_00010241_00010752_0024_0014	19.5
81	20071026_00005_003_rg_00010241_00010752_0024_0014	19.5
82	20071026_00005_004_rg_00010241_00010752_0024_0014	19.5
83	20071026_00005_005_rg_00010241_00010752_0024_0014	19.5
84	20071026_00005_006_rg_00010241_00010752_0024_0014	19.5
85	20071026_00006_001_rg_00010241_00010752_0024_0014	19

86	20071026_00006_002_rg_00010241_00010752_0024_0014	19
87	20071026_00006_003_rg_00010241_00010752_0024_0014	19
88	20071026_00006_004_rg_00010241_00010752_0024_0014	19
89	20071026_00006_005_rg_00010241_00010752_0024_0014	19
90	20071026_00006_006_rg_00010241_00010752_0024_0014	19
91	20071026_00007_001_rg_00010241_00010752_0024_0014	20
92	20071026_00007_002_rg_00010241_00010752_0024_0014	20
93	20071026_00007_003_rg_00010241_00010752_0024_0014	19.5
94	20071026_00007_004_rg_00010241_00010752_0024_0014	20
95	20071026_00007_005_rg_00010241_00010752_0024_0014	20
96	20071026_00007_006_rg_00010241_00010752_0024_0014	20
97	20071026_00008_001_rg_00010241_00010752_0024_0014	18.5
98	20071026_00008_002_rg_00010241_00010752_0024_0014	18.5
99	20071026_00008_003_rg_00010241_00010752_0024_0014	18.5
100	20071026_00008_004_rg_00010241_00010752_0024_0014	18.5
101	20071026_00008_005_rg_00010241_00010752_0024_0014	19
102	20071026_00008_006_rg_00010241_00010752_0024_0014	18.5
103	20071026_00009_001_rg_00010241_00010752_0024_0014	19
104	20071026_00009_002_rg_00010241_00010752_0024_0014	19
105	20071026_00009_003_rg_00010241_00010752_0024_0014	19
106	20071026_00009_004_rg_00010241_00010752_0024_0014	19
107	20071026_00009_005_rg_00010241_00010752_0024_0014	19
108	20071026_00009_006_rg_00010241_00010752_0024_0014	19
109	20071112_00010_001_rg_00010241_00010752_0024_0014	19.5
110	20071112_00010_002_rg_00010241_00010752_0024_0014	20
111	20071112_00010_003_rg_00010241_00010752_0024_0014	19.5
112	20071112_00010_004_rg_00010241_00010752_0024_0014	19.5
113	20071112_00010_005_rg_00010241_00010752_0024_0014	19.5
114	20071112_00010_006_rg_00010241_00010752_0024_0014	19.5
115	20071112_00011_001_rg_00010241_00010752_0024_0014	18.5
116	20071112_00011_002_rg_00010241_00010752_0024_0014	19
117	20071112_00011_003_rg_00010241_00010752_0024_0014	19
118	20071112_00011_004_rg_00010241_00010752_0024_0014	19
119	20071112_00011_005_rg_00010241_00010752_0024_0014	18.5
120	20071112_00011_006_rg_00010241_00010752_0024_0014	18.5
121	20071019_00002_001_rg_00010241_00010752_0027_0037	31
122	20071019_00002_002_rg_00010241_00010752_0027_0037	31
123	20071019_00002_003_rg_00010241_00010752_0027_0037	31
124	20071019_00002_004_rg_00010241_00010752_0027_0037	31
125	20071019_00002_005_rg_00010241_00010752_0027_0037	33
126	20071019_00002_006_rg_00010241_00010752_0027_0037	31
127	20071019_00003_001_rg_00010241_00010752_0027_0037	31
128	20071019_00003_002_rg_00010241_00010752_0027_0037	31.5
129	20071019_00003_003_rg_00010241_00010752_0027_0037	31
130	20071019_00003_004_rg_00010241_00010752_0027_0037	31
131	20071019_00003_005_rg_00010241_00010752_0027_0037	31
132	20071019_00003_006_rg_00010241_00010752_0027_0037	31

133	20071025_00004_001_rg_00010241_00010752_0027_0037	32
134	20071025_00004_002_rg_00010241_00010752_0027_0037	32
135	20071025_00004_003_rg_00010241_00010752_0027_0037	32
136	20071025_00004_004_rg_00010241_00010752_0027_0037	32
137	20071025_00004_005_rg_00010241_00010752_0027_0037	32
138	20071025_00004_006_rg_00010241_00010752_0027_0037	32
139	20071026_00005_001_rg_00010241_00010752_0027_0037	30
140	20071026_00005_002_rg_00010241_00010752_0027_0037	31
141	20071026_00005_003_rg_00010241_00010752_0027_0037	30.5
142	20071026_00005_004_rg_00010241_00010752_0027_0037	30.5
143	20071026_00005_005_rg_00010241_00010752_0027_0037	30.5
144	20071026_00005_006_rg_00010241_00010752_0027_0037	30.5
145	20071026_00006_001_rg_00010241_00010752_0027_0037	31
146	20071026_00006_002_rg_00010241_00010752_0027_0037	30.5
147	20071026_00006_003_rg_00010241_00010752_0027_0037	30.5
148	20071026_00006_004_rg_00010241_00010752_0027_0037	31
149	20071026_00006_005_rg_00010241_00010752_0027_0037	30.5
150	20071026_00006_006_rg_00010241_00010752_0027_0037	30.5
151	20071026_00007_001_rg_00010241_00010752_0027_0037	30
152	20071026_00007_002_rg_00010241_00010752_0027_0037	30
153	20071026_00007_003_rg_00010241_00010752_0027_0037	30.5
154	20071026_00007_004_rg_00010241_00010752_0027_0037	30
155	20071026_00007_005_rg_00010241_00010752_0027_0037	30
156	20071026_00007_006_rg_00010241_00010752_0027_0037	30
157	20071026_00008_001_rg_00010241_00010752_0027_0037	31
158	20071026_00008_002_rg_00010241_00010752_0027_0037	31
159	20071026_00008_003_rg_00010241_00010752_0027_0037	31
160	20071026_00008_004_rg_00010241_00010752_0027_0037	31
161	20071026_00008_005_rg_00010241_00010752_0027_0037	31
162	20071026_00008_006_rg_00010241_00010752_0027_0037	31
163	20071026_00009_001_rg_00010241_00010752_0027_0037	31
164	20071026_00009_002_rg_00010241_00010752_0027_0037	30.5
165	20071026_00009_003_rg_00010241_00010752_0027_0037	31
166	20071026_00009_004_rg_00010241_00010752_0027_0037	30.5
167	20071026_00009_005_rg_00010241_00010752_0027_0037	31
168	20071026_00009_006_rg_00010241_00010752_0027_0037	30.5
169	20071112_00010_001_rg_00010241_00010752_0027_0037	30
170	20071112_00010_002_rg_00010241_00010752_0027_0037	30
171	20071112_00010_003_rg_00010241_00010752_0027_0037	30
172	20071112_00010_004_rg_00010241_00010752_0027_0037	30
173	20071112_00010_005_rg_00010241_00010752_0027_0037	30
174	20071112_00010_006_rg_00010241_00010752_0027_0037	30
175	20071112_00011_001_rg_00010241_00010752_0027_0037	31
176	20071112_00011_002_rg_00010241_00010752_0027_0037	31
177	20071112_00011_003_rg_00010241_00010752_0027_0037	31
178	20071112_00011_004_rg_00010241_00010752_0027_0037	31
179	20071112_00011_005_rg_00010241_00010752_0027_0037	31

180	20071112_00011_006_rg_00010241_00010752_0027_0037	31
181	20071019_00002_001_rg_00010241_00010752_0050_0040	45
182	20071019_00002_002_rg_00010241_00010752_0050_0040	45
183	20071019_00002_003_rg_00010241_00010752_0050_0040	45
184	20071019_00002_004_rg_00010241_00010752_0050_0040	45
185	20071019_00002_005_rg_00010241_00010752_0050_0040	45
186	20071019_00002_006_rg_00010241_00010752_0050_0040	45
187	20071019_00003_001_rg_00010241_00010752_0050_0040	45.5
188	20071019_00003_002_rg_00010241_00010752_0050_0040	45
189	20071019_00003_003_rg_00010241_00010752_0050_0040	45
190	20071019_00003_004_rg_00010241_00010752_0050_0040	45
191	20071019_00003_005_rg_00010241_00010752_0050_0040	45
192	20071019_00003_006_rg_00010241_00010752_0050_0040	45
193	20071025_00004_001_rg_00010241_00010752_0050_0040	44
194	20071025_00004_002_rg_00010241_00010752_0050_0040	44
195	20071025_00004_003_rg_00010241_00010752_0050_0040	44
196	20071025_00004_004_rg_00010241_00010752_0050_0040	44
197	20071025_00004_005_rg_00010241_00010752_0050_0040	44
198	20071025_00004_006_rg_00010241_00010752_0050_0040	44
199	20071026_00005_001_rg_00010241_00010752_0050_0040	46
200	20071026_00005_002_rg_00010241_00010752_0050_0040	46
201	20071026_00005_003_rg_00010241_00010752_0050_0040	46
202	20071026_00005_004_rg_00010241_00010752_0050_0040	46
203	20071026_00005_005_rg_00010241_00010752_0050_0040	46
204	20071026_00005_006_rg_00010241_00010752_0050_0040	46
205	20071026_00006_001_rg_00010241_00010752_0050_0040	45.5
206	20071026_00006_002_rg_00010241_00010752_0050_0040	46
207	20071026_00006_003_rg_00010241_00010752_0050_0040	45.5
208	20071026_00006_004_rg_00010241_00010752_0050_0040	46
209	20071026_00006_005_rg_00010241_00010752_0050_0040	46
210	20071026_00006_006_rg_00010241_00010752_0050_0040	46
211	20071026_00007_001_rg_00010241_00010752_0050_0040	46
212	20071026_00007_002_rg_00010241_00010752_0050_0040	46
213	20071026_00007_003_rg_00010241_00010752_0050_0040	46
214	20071026_00007_004_rg_00010241_00010752_0050_0040	46
215	20071026_00007_005_rg_00010241_00010752_0050_0040	46
216	20071026_00007_006_rg_00010241_00010752_0050_0040	46
217	20071026_00008_001_rg_00010241_00010752_0050_0040	45
218	20071026_00008_002_rg_00010241_00010752_0050_0040	45
219	20071026_00008_003_rg_00010241_00010752_0050_0040	45
220	20071026_00008_004_rg_00010241_00010752_0050_0040	45
221	20071026_00008_005_rg_00010241_00010752_0050_0040	45
222	20071026_00008_006_rg_00010241_00010752_0050_0040	45
223	20071026_00009_001_rg_00010241_00010752_0050_0040	46
224	20071026_00009_002_rg_00010241_00010752_0050_0040	46
225	20071026_00009_003_rg_00010241_00010752_0050_0040	46
226	20071026_00009_004_rg_00010241_00010752_0050_0040	46

227	20071026_00009_005_rg_00010241_00010752_0050_0040	46
228	20071026_00009_006_rg_00010241_00010752_0050_0040	46
229	20071112_00010_001_rg_00010241_00010752_0050_0040	47
230	20071112_00010_002_rg_00010241_00010752_0050_0040	47
231	20071112_00010_003_rg_00010241_00010752_0050_0040	47
232	20071112_00010_004_rg_00010241_00010752_0050_0040	47
233	20071112_00010_005_rg_00010241_00010752_0050_0040	47
234	20071112_00010_006_rg_00010241_00010752_0050_0040	47
235	20071112_00011_001_rg_00010241_00010752_0050_0040	45
236	20071112_00011_002_rg_00010241_00010752_0050_0040	45.5
237	20071112_00011_003_rg_00010241_00010752_0050_0040	45.5
238	20071112_00011_004_rg_00010241_00010752_0050_0040	45.5
239	20071112_00011_005_rg_00010241_00010752_0050_0040	45
240	20071112_00011_006_rg_00010241_00010752_0050_0040	45
241	20071019_00002_001_rg_00010241_00010752_0053_0063	56.5
242	20071019_00002_002_rg_00010241_00010752_0053_0063	56
243	20071019_00002_003_rg_00010241_00010752_0053_0063	56
244	20071019_00002_004_rg_00010241_00010752_0053_0063	56
245	20071019_00002_005_rg_00010241_00010752_0053_0063	56
246	20071019_00002_006_rg_00010241_00010752_0053_0063	57
247	20071019_00003_001_rg_00010241_00010752_0053_0063	56
248	20071019_00003_002_rg_00010241_00010752_0053_0063	56
249	20071019_00003_003_rg_00010241_00010752_0053_0063	56
250	20071019_00003_004_rg_00010241_00010752_0053_0063	56
251	20071019_00003_005_rg_00010241_00010752_0053_0063	56.5
252	20071019_00003_006_rg_00010241_00010752_0053_0063	56
253	20071025_00004_001_rg_00010241_00010752_0053_0063	58
254	20071025_00004_002_rg_00010241_00010752_0053_0063	58
255	20071025_00004_003_rg_00010241_00010752_0053_0063	58
256	20071025_00004_004_rg_00010241_00010752_0053_0063	58
257	20071025_00004_005_rg_00010241_00010752_0053_0063	58
258	20071025_00004_006_rg_00010241_00010752_0053_0063	58
259	20071026_00005_001_rg_00010241_00010752_0053_0063	56
260	20071026_00005_002_rg_00010241_00010752_0053_0063	55.5
261	20071026_00005_003_rg_00010241_00010752_0053_0063	56
262	20071026_00005_004_rg_00010241_00010752_0053_0063	55.5
263	20071026_00005_005_rg_00010241_00010752_0053_0063	56
264	20071026_00005_006_rg_00010241_00010752_0053_0063	55.5
265	20071026_00006_001_rg_00010241_00010752_0053_0063	56
266	20071026_00006_002_rg_00010241_00010752_0053_0063	56
267	20071026_00006_003_rg_00010241_00010752_0053_0063	56
268	20071026_00006_004_rg_00010241_00010752_0053_0063	56
269	20071026_00006_005_rg_00010241_00010752_0053_0063	56
270	20071026_00006_006_rg_00010241_00010752_0053_0063	56
271	20071026_00007_001_rg_00010241_00010752_0053_0063	56
272	20071026_00007_002_rg_00010241_00010752_0053_0063	56
273	20071026_00007_003_rg_00010241_00010752_0053_0063	56

274	20071026_00007_004_rg_00010241_00010752_0053_0063	56
275	20071026_00007_005_rg_00010241_00010752_0053_0063	56
276	20071026_00007_006_rg_00010241_00010752_0053_0063	56
277	20071026_00008_001_rg_00010241_00010752_0053_0063	56
278	20071026_00008_002_rg_00010241_00010752_0053_0063	56.5
279	20071026_00008_003_rg_00010241_00010752_0053_0063	56.5
280	20071026_00008_004_rg_00010241_00010752_0053_0063	56.5
281	20071026_00008_005_rg_00010241_00010752_0053_0063	56.5
282	20071026_00008_006_rg_00010241_00010752_0053_0063	56.5
283	20071026_00009_001_rg_00010241_00010752_0053_0063	56
284	20071026_00009_002_rg_00010241_00010752_0053_0063	56
285	20071026_00009_003_rg_00010241_00010752_0053_0063	56
286	20071026_00009_004_rg_00010241_00010752_0053_0063	56
287	20071026_00009_005_rg_00010241_00010752_0053_0063	56
288	20071026_00009_006_rg_00010241_00010752_0053_0063	56
289	20071112_00010_001_rg_00010241_00010752_0053_0063	54.5
290	20071112_00010_002_rg_00010241_00010752_0053_0063	54.5
291	20071112_00010_003_rg_00010241_00010752_0053_0063	54.5
292	20071112_00010_004_rg_00010241_00010752_0053_0063	54.5
293	20071112_00010_005_rg_00010241_00010752_0053_0063	54.5
294	20071112_00010_006_rg_00010241_00010752_0053_0063	54.5
295	20071112_00011_001_rg_00010241_00010752_0053_0063	56.5
296	20071112_00011_002_rg_00010241_00010752_0053_0063	56
297	20071112_00011_003_rg_00010241_00010752_0053_0063	56
298	20071112_00011_004_rg_00010241_00010752_0053_0063	56
299	20071112_00011_005_rg_00010241_00010752_0053_0063	56
300	20071112_00011_006_rg_00010241_00010752_0053_0063	56.5

Appendix V: List number 5. part one. Epochs' list extracted using customized software. Matrix was positioned by operator A. Each epoch is identified by a code containing the following details: date of acquisition, subject ID, contraction number, operator code, signal epoch, matrix column. Operator A collected all epochs included in this list. A1, 1st estimate by operator A.

n	Epochs_ID	A1
1	20071019_00002_002_mb_00010241_00010752_0001_0011	6
2	20071019_00002_002_mb_00010241_00010752_0024_0014	18
3	20071019_00002_002_mb_00010241_00010752_0027_0037	31.5
4	20071019_00002_002_mb_00010241_00010752_0050_0040	45
5	20071019_00002_002_mb_00010241_00010752_0053_0063	57
6	20071019_00003_003_mb_00010241_00010752_0001_0011	6
7	20071019_00003_003_mb_00010241_00010752_0024_0014	18.5
8	20071019_00003_003_mb_00010241_00010752_0027_0037	31
9	20071019_00003_003_mb_00010241_00010752_0050_0040	46
10	20071019_00003_003_mb_00010241_00010752_0053_0063	56
11	20071025_00004_001_mb_00010241_00010752_0001_0011	4.5
12	20071025_00004_001_mb_00010241_00010752_0024_0014	19
13	20071025_00004_001_mb_00010241_00010752_0027_0037	31.5
14	20071025_00004_001_mb_00010241_00010752_0050_0040	44
15	20071025_00004_001_mb_00010241_00010752_0053_0063	57.5
16	20071026_00005_001_mb_00010241_00010752_0001_0011	5
17	20071026_00005_001_mb_00010241_00010752_0024_0014	18.5
18	20071026_00005_001_mb_00010241_00010752_0027_0037	31.5
19	20071026_00005_001_mb_00010241_00010752_0050_0040	45
20	20071026_00005_001_mb_00010241_00010752_0053_0063	57
21	20071026_00006_003_mb_00010241_00010752_0001_0011	5
22	20071026_00006_003_mb_00010241_00010752_0024_0014	19
23	20071026_00006_003_mb_00010241_00010752_0027_0037	30
24	20071026_00006_003_mb_00010241_00010752_0050_0040	46
25	20071026_00006_003_mb_00010241_00010752_0053_0063	56
26	20071026_00007_001_mb_00010241_00010752_0001_0011	4
27	20071026_00007_001_mb_00010241_00010752_0024_0014	19.5
28	20071026_00007_001_mb_00010241_00010752_0027_0037	30.5
29	20071026_00007_001_mb_00010241_00010752_0050_0040	46
30	20071026_00007_001_mb_00010241_00010752_0053_0063	56
31	20071026_00008_001_mb_00010241_00010752_0001_0011	5
32	20071026_00008_001_mb_00010241_00010752_0024_0014	19
33	20071026_00008_001_mb_00010241_00010752_0027_0037	30
34	20071026_00008_001_mb_00010241_00010752_0050_0040	46
35	20071026_00008_001_mb_00010241_00010752_0053_0063	56
36	20071026_00009_001_mb_00010241_00010752_0001_0011	6
37	20071026_00009_001_mb_00010241_00010752_0024_0014	18
38	20071026_00009_001_mb_00010241_00010752_0027_0037	32

39	20071026_00009_001_mb_00010241_00010752_0050_0040	45
40	20071026_00009_001_mb_00010241_00010752_0053_0063	57
41	20071112_00010_002_mb_00010241_00010752_0001_0011	4.5
42	20071112_00010_002_mb_00010241_00010752_0024_0014	19.5
43	20071112_00010_002_mb_00010241_00010752_0027_0037	30.5
44	20071112_00010_002_mb_00010241_00010752_0050_0040	47
45	20071112_00010_002_mb_00010241_00010752_0053_0063	55
46	20071112_00011_001_mb_00010241_00010752_0001_0011	5
47	20071112_00011_001_mb_00010241_00010752_0024_0014	19
48	20071112_00011_001_mb_00010241_00010752_0027_0037	31
49	20071112_00011_001_mb_00010241_00010752_0050_0040	45.5
50	20071112_00011_001_mb_00010241_00010752_0053_0063	56

Appendix VI: List number 5, part two. Epochs' list extracted using customized software. Matrix was positioned by operator B. Each epoch is identified by a code containing the following details: date of acquisition, subject ID, contraction number, operator code, signal epoch, matrix column. Operator B collected all epochs included in this list. B1, 1st estimate by operator B.

n	Epochs_ID	B1
1	20071019_00002_002_rg_00010241_00010752_0001_0011	5.5
2	20071019_00002_002_rg_00010241_00010752_0024_0014	18.5
3	20071019_00002_002_rg_00010241_00010752_0027_0037	31
4	20071019_00002_002_rg_00010241_00010752_0050_0040	45
5	20071019_00002_002_rg_00010241_00010752_0053_0063	56
6	20071019_00003_001_rg_00010241_00010752_0001_0011	5
7	20071019_00003_001_rg_00010241_00010752_0024_0014	19
8	20071019_00003_001_rg_00010241_00010752_0027_0037	31
9	20071019_00003_001_rg_00010241_00010752_0050_0040	45.5
10	20071019_00003_001_rg_00010241_00010752_0053_0063	56
11	20071025_00004_001_rg_00010241_00010752_0001_0011	5
12	20071025_00004_001_rg_00010241_00010752_0024_0014	18
13	20071025_00004_001_rg_00010241_00010752_0027_0037	32
14	20071025_00004_001_rg_00010241_00010752_0050_0040	44
15	20071025_00004_001_rg_00010241_00010752_0053_0063	58
16	20071026_00005_001_rg_00010241_00010752_0001_0011	4.5
17	20071026_00005_001_rg_00010241_00010752_0024_0014	19.5
18	20071026_00005_001_rg_00010241_00010752_0027_0037	30
19	20071026_00005_001_rg_00010241_00010752_0050_0040	46
20	20071026_00005_001_rg_00010241_00010752_0053_0063	56
21	20071026_00006_002_rg_00010241_00010752_0001_0011	5
22	20071026_00006_002_rg_00010241_00010752_0024_0014	19
23	20071026_00006_002_rg_00010241_00010752_0027_0037	30.5
24	20071026_00006_002_rg_00010241_00010752_0050_0040	46
25	20071026_00006_002_rg_00010241_00010752_0053_0063	56
26	20071026_00007_001_rg_00010241_00010752_0001_0011	4
27	20071026_00007_001_rg_00010241_00010752_0024_0014	20
28	20071026_00007_001_rg_00010241_00010752_0027_0037	30
29	20071026_00007_001_rg_00010241_00010752_0050_0040	46
30	20071026_00007_001_rg_00010241_00010752_0053_0063	56
31	20071026_00008_002_rg_00010241_00010752_0001_0011	5.5
32	20071026_00008_002_rg_00010241_00010752_0024_0014	18.5
33	20071026_00008_002_rg_00010241_00010752_0027_0037	31
34	20071026_00008_002_rg_00010241_00010752_0050_0040	45
35	20071026_00008_002_rg_00010241_00010752_0053_0063	56.5
36	20071026_00009_001_rg_00010241_00010752_0001_0011	5.5
37	20071026_00009_001_rg_00010241_00010752_0024_0014	19

38	20071026_00009_001_rg_00010241_00010752_0027_0037	31
39	20071026_00009_001_rg_00010241_00010752_0050_0040	46
40	20071026_00009_001_rg_00010241_00010752_0053_0063	56
41	20071112_00010_001_rg_00010241_00010752_0001_0011	4
42	20071112_00010_001_rg_00010241_00010752_0024_0014	19.5
43	20071112_00010_001_rg_00010241_00010752_0027_0037	30
44	20071112_00010_001_rg_00010241_00010752_0050_0040	47
45	20071112_00010_001_rg_00010241_00010752_0053_0063	54.5
46	20071112_00011_002_rg_00010241_00010752_0001_0011	5.5
47	20071112_00011_002_rg_00010241_00010752_0024_0014	19
48	20071112_00011_002_rg_00010241_00010752_0027_0037	31
49	20071112_00011_002_rg_00010241_00010752_0050_0040	45.5
50	20071112_00011_002_rg_00010241_00010752_0053_0063	56

Appendix VII: Sample size table for Cohen's Kappa statistic. Minimum number of estimates required to detect a kappa coefficient as statistically significant ($p < 0.05$) in a 2-rater study.

		1-Tailed Test Null Value=.00		2-Tailed Test Null Value=.00	
Proportion of positive ratings	**Kappa to detect**	**n at 80% power**	**n at 90% power**	**n at 80% power**	**n at 90% power**
.10	.40	39	54	50	66
.30	.40	39	54	50	66
.50	.40	39	54	50	66
.70	.40	39	54	50	66
.90	.40	39	54	50	66
.10	.50	25	35	32	43
.30	.50	25	35	32	43
.50	.50	25	35	32	43
.70	.50	25	35	32	43
.90	.50	25	35	32	43
.10	.60	18	24	22	30
.30	.60	18	24	22	30
.50	.60	18	24	22	30
.70	.60	18	24	22	30
.90	.60	13	18	17	22
.10	.70	13	18	17	22
.30	.70	13	18	17	22
.50	.70	13	18	17	22
.70	.70	13	18	17	22
.90	.70	13	18	17	22
.10	.80	10	14	13	17
.30	.80	10	14	13	17
.50	.80	10	14	13	17
.70	.80	10	14	13	17
.90	.80	10	14	13	17
.10	.90	8	11	10	13
.30	.90	8	11	10	13
.50	.90	8	11	10	13
.70	.90	8	11	10	13
.90	.90	8	11	10	13

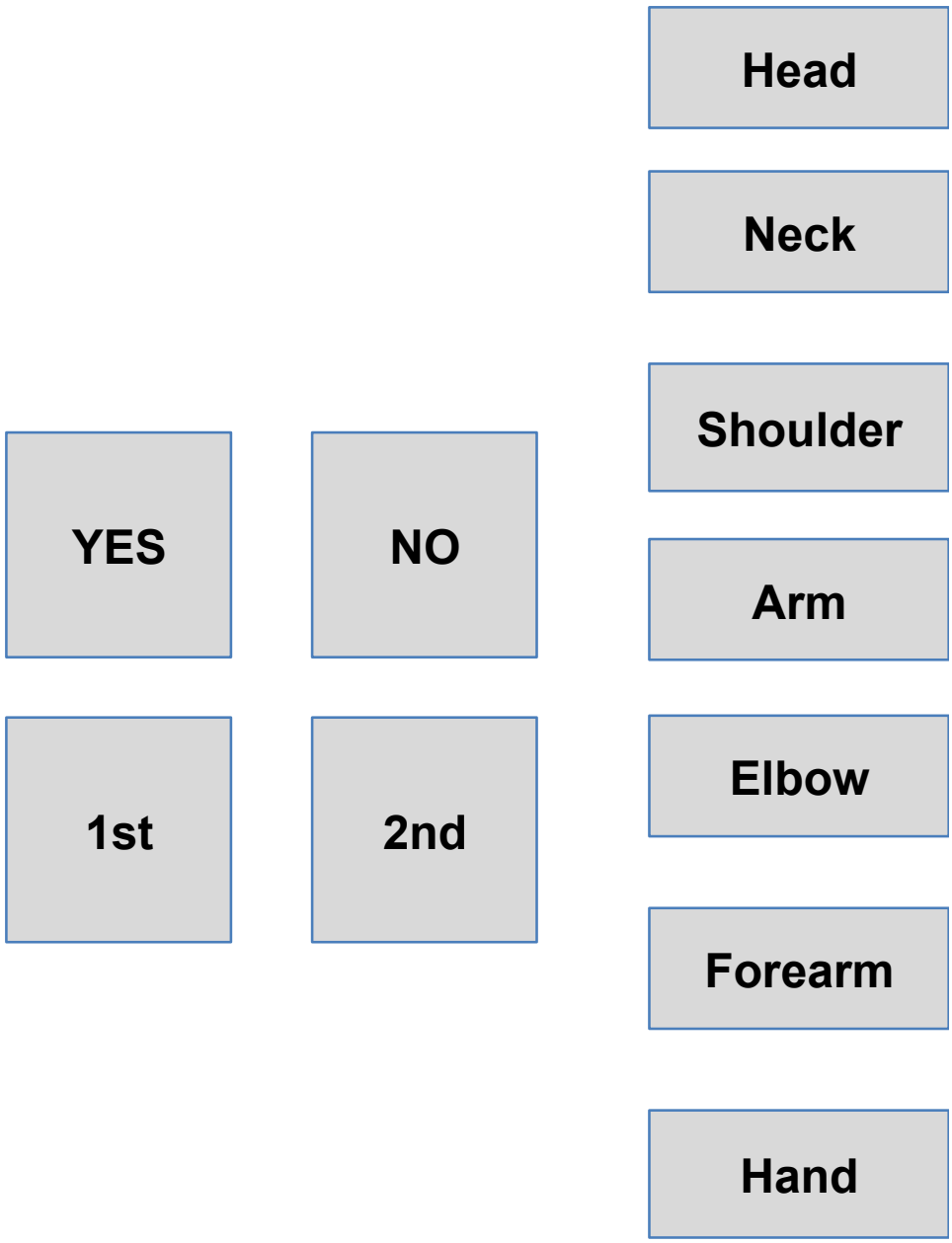
Appendix VIII: Operators' and contractions' order during the data collecting. Randomization has been performed prior to the experimental sessions.

Subject_ID	Operator	Contraction order (%MVC)						Operator	Contraction order (%MVC)					
1	A	40	20	20	20	40	40	B	40	20	40	20	40	20
2	A	40	40	20	40	20	20	B	20	40	20	40	40	20
3	A	20	40	20	20	40	40	B	20	40	20	40	20	40
4	A	20	20	40	40	20	40	B	20	20	40	20	40	40
5	B	40	20	40	20	20	40	A	40	40	20	40	20	20
6	A	20	20	20	40	40	40	B	20	20	40	20	40	40
7	A	20	20	40	40	40	20	B	40	20	40	40	20	20
8	B	20	40	20	20	40	40	A	20	40	20	20	40	40
9	A	40	20	20	40	20	40	B	20	20	40	20	40	40
10	B	40	20	20	20	40	40	A	20	20	40	40	40	20

Appendix IX: Checklist for reliability studies.

Items	Item description (abbreviated)
1	Was the sample of subjects representative?
2	Were raters blinded to the findings of other raters?
3	Were raters blinded to the findings of other raters?
4	Were raters blinded to their own prior findings?
5	Were raters blinded to the accepted reference standard?
6	Were raters blinded to clinical information not part of test
7	Were raters blinded to additional non-clinical cues?
8	Was the order of examination varied?
9	Was the time interval between repeated measures appropriate?
10	Was the test applied correctly and interpreted appropriately?
11	Were appropriate statistical measures of agreement used?

Appendix X: Pre-set answers for MTrP' palpation. The following questions was asked: is this spot unusually painful?; I will compress two spots, a first one and second one. Please tell me which is the most painful; do you recognize this pain as a familiar complaint?; Does the pain occurs anywhere from the spot that I am compressing? If yes, indicate where according to the anatomical regions reported on the sheet.



Appendix XI Table for sample size' computation.

ρ_o	ρ_1								
n=2	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1		591.2	142.8	60.6	32.2	19.1	12	7.7	4.8
0.2			543.7	128.2	53	27.2	15.5	9.2	5.3
0.3				476.2	109	43.5	21.4	11.4	6.1
0.4					393	86.6	32.9	15.1	7.1
0.5						300.3	62.6	22	8.8
0.6							205.4	39.1	11.7
0.7								117.1	18.4
0.8									45.8

Appendix XII: Results of the normality for tests Chapter 4. X1, X value measured during the first palpatory examination; X2, X value measured during the second palpatory examination. Y1, Y value measured during the first palpatory examination; Y2, Y value measured during the second palpatory examination; MTrP, distance between MTrP_1 and MTrP_2, L, left; R, right.

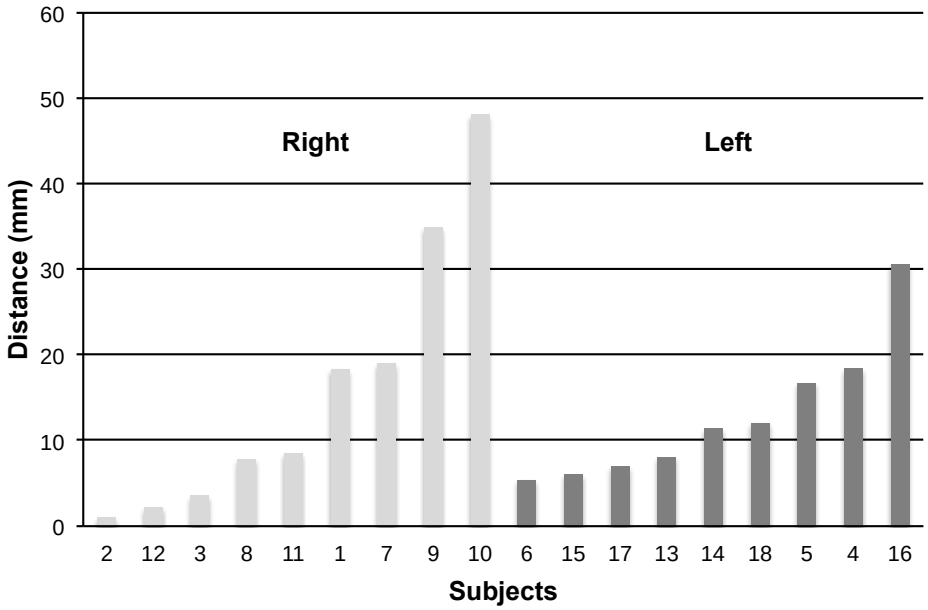
	Shapiro-Wilk		
	Statistic	df	Sig.
X1	.963	24	.494
Y1	.928	24	.089
X2	.958	24	.401
Y2	.931	24	.103

		Shapiro-Wilk		
	SIDE	Statistic	df	Sig.
MTrP_d	L	.910	12	.212
	R	.908	12	.203
X1	L	.938	12	.471
	R	.980	12	.983
Y1	L	.839	12	.027
	R	.832	12	.022

Appendix XIII: Results of the palpation' procedures for the enrolled subjects**.**

Subjects	MTrP Diagnostic criteria				
	Taut band	Spot tenderness	Pain recognition	Referred pain	Referral zone
1	x	x	x		
2	x	x	x	x	Head
3	x	x	x		
4	x	x	x	x	Neck
5	x	x	x	x	Shoulder
6	x	x	x	x	Neck
7	x	x	x		
8	x	x	x	x	Neck
9	x	x	x	x	Head
10	x	x	x		
11	x	x	x		
12	x	x	x		
13	x	x	x		
14	x	x	x	x	Head
15	x	x	x		
16	x	x	x	x	Arm/elbow
17	x	x	x		
18	x	x	x		
19	x	x	x	x	Head
20	x	x	x		
21	x	x	x	x	Shoulder
22	x	x	x	x	Neck
23	x	x	x	x	Neck
24	x	x	x		

Appendix XIV: The distance between MTrP_1 and MTrP_2 for each subject. MTrP_1, myofascial trigger point detected during the first palpatory examination; MTrP_2, myofascial trigger point detected during the second palpatory examination.



Appendix XV: NDI – Italian version.

Neck Disability Index – Versione Italiana (Monticone at al. 2012).

ISTRUZIONI: Il presente questionario è stato creato per permetterci di capire in che modo il dolore che prova al collo abbia condizionato la Sua capacità di gestire le attività della vita quotidiana. Per cortesia, risponda ad ogni sezione barrando LA RISPOSTA che giudica più pertinente. Ci rendiamo conto che si possa trovare d'accordo con più di una affermazione, ma PROVI GENTILMENTE A CERCHIARE SOLO LA RISPOSTA CHE DESCRIVE MAGGIORMENTE IL SUO PROBLEMA PROPRIO ADESSO.

Sezione 1 - Intensità del dolore

□ Al momento non ho dolore cervicale.
□ Al momento il dolore cervicale è molto lieve.
□ Al momento il dolore cervicale è di media intensità.
□ Al momento il dolore cervicale è abbastanza forte.
□ Al momento il dolore cervicale è molto forte.
□ Al momento il dolore cervicale è il massimo immaginabile.

Sezione 2 - Cura personale (lavarsi, vestirsi, ecc.)

□ Riesco a prendermi cura di me stesso/a normalmente senza sentire più dolore cervicale del solito.
□ Riesco a prendermi cura di me stesso/a normalmente ma mi causa più dolore cervicale del solito.
□ Mi fa male prendermi cura di me stesso/a e sono lento/a e prudente.
□ Ho bisogno di un po' di aiuto ma riesco per lo più a prendermi cura di me stesso/a.
□ Ho bisogno di aiuto ogni giorno in quasi tutti gli aspetti della cura di me stesso/a.
□ Non mi vesto, mi lavo con difficoltà e sto a letto.

Sezione 3 - Alzare pesi

□ Riesco a sollevare oggetti pesanti senza sentire più dolore cervicale del solito.
□ Riesco a sollevare oggetti pesanti ma sentendo più dolore cervicale del solito.
□ Il dolore cervicale mi impedisce di sollevare oggetti pesanti da terra, ma ci riesco se sono posizionati in maniera opportuna, per esempio su un tavolo.
□ Il dolore cervicale mi impedisce di sollevare oggetti pesanti, ma riesco a sollevare oggetti leggeri o di medio peso se sono posizionati in maniera opportuna.
□ Riesco a sollevare solo oggetti molto leggeri.
□ Non riesco a sollevare o trasportare assolutamente niente.

Sezione 4 – Leggere

□ Riesco a leggere quanto voglio senza provare alcun dolore al collo.
□ Riesco a leggere quanto voglio avvertendo un dolore al collo lieve.
□ Riesco a leggere quanto voglio avvertendo un dolore al collo di media intensità.
□ Non riesco a leggere quanto voglio a causa di un dolore al collo di media intensità.
□ Non riesco a leggere quanto voglio a causa di un dolore al collo molto forte.
□ Non riesco a leggere del tutto.

Sezione 5 – Mal di testa

□ Non provo mal di testa per nulla.
□ Provo un mal di testa lieve che insorge raramente.
□ Provo un mal di testa di media intensità che insorge raramente.
□ Provo un mal di testa di media intensità che insorge frequentemente.
□ Provo un mal di testa molto forte che insorge frequentemente.
□ Provo quasi sempre mal di testa.

Sezione 6 – Concentrarsi

□ Riesco a concentrarmi perfettamente quando lo desidero senza difficoltà.
□ Riesco a concentrarmi perfettamente quando lo desidero con leggera difficoltà.
□ Avverto una difficoltà intermedia a concentrarmi quando lo desidero.
□ Avverto molta difficoltà a concentrarmi quando lo desidero.
□ Avverto moltissima difficoltà a concentrarmi quando lo desidero.
□ Non riesco a concentrarmi del tutto.

Sezione 7 – Lavorare

□ Riesco a svolgere tutto il lavoro che voglio.
□ Riesco a svolgere solo il mio lavoro abituale, ma nulla di più.
□ Riesco a svolgere parte del mio lavoro abituale, ma nulla di più.
□ Non riesco a svolgere il mio lavoro abituale.
□ Svolgo ogni lavoro con molta difficoltà.
□ Non riesco più a svolgere alcun lavoro.

Sezione 8 - Guidare

□ Riesco a guidare la mia macchina senza alcun dolore al collo.
□ Riesco a guidare la mia macchina fin quando voglio provando un lieve dolore al collo.
□ Riesco a guidare la mia macchina fin quando voglio provando un dolore al collo di media intensità.
□ Non riesco a guidare la mia macchina fin quando voglio a causa di un dolore al collo di media intensità.
□ Riesco a guidare proprio con molta difficoltà a causa di un forte dolore al collo.

□ Non riesco più a guidare la mia macchina a causa del dolore cervicale.

Sezione 9 – Dormire

□ Non ho problemi per dormire.
□ Il mio riposo è scarsamente disturbato (meno di un'ora di insonnia).
□ Il mio riposo è leggermente disturbato (1-2 ore di insonnia).
□ Il mio riposo è moderatamente disturbato (2-3 ore di insonnia).
□ Il mio riposo è disturbato moltissimo (3-5 ore di insonnia).
□ Il mio riposo è completamente disturbato (5-7 ore di insonnia).

Sezione 10 – Svagarsi

□ Posso dedicarmi a tutti i miei passatempi senza alcun dolore al collo.
□ Posso dedicarmi a tutti i miei passatempi con un po' di dolore al mio collo.
□ Posso dedicarmi a molti, ma non a tutti i miei passatempi a causa del dolore al mio collo.
□ Posso dedicarmi solo ad alcuni dei miei passatempi a causa del dolore al mio collo.
□ Posso dedicarmi con difficoltà ai miei passatempi a causa del dolore al mio collo.
□ Non riesco più a dedicarmi a nessun passatempo.

Commenti:……
……
……
……
Nominativo:
……
.
Data di compilazione:
……
Punteggio:
……
…

Calcolo del Punteggio per il Neck Disability Index.

1. Ognuna delle 10 sezioni ottiene un punteggio separato (da 0 a 5) che è poi sommato (totale max. = 50).

ESEMPIO:

Sezione 1 - Intensità del dolore

	Punteggio
A. Al momento non ho dolore cervicale.	0
B. Al momento il dolore cervicale è molto lieve.	1
C. Al momento il dolore cervicale è di media intensità.	2
D. Al momento il dolore cervicale è abbastanza forte.	3
E. Al momento il dolore cervicale è molto forte.	4
F. Al momento il dolore cervicale è il massimo immaginabile.	5

2. Se tutte le sezioni sono state compilate, raddoppiare semplicemente il punteggio ottenuto.

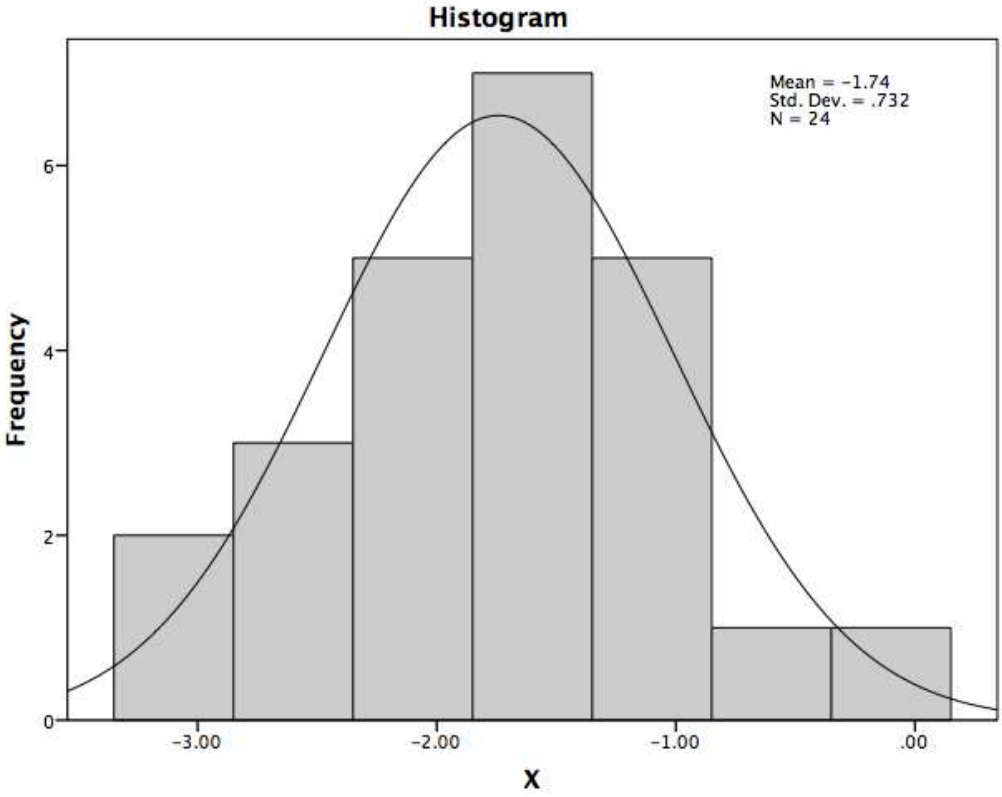
3. Se una sezione è stata tralasciata, dividere il punteggio totale per il numero delle sezioni compilate, moltiplicando per 5.

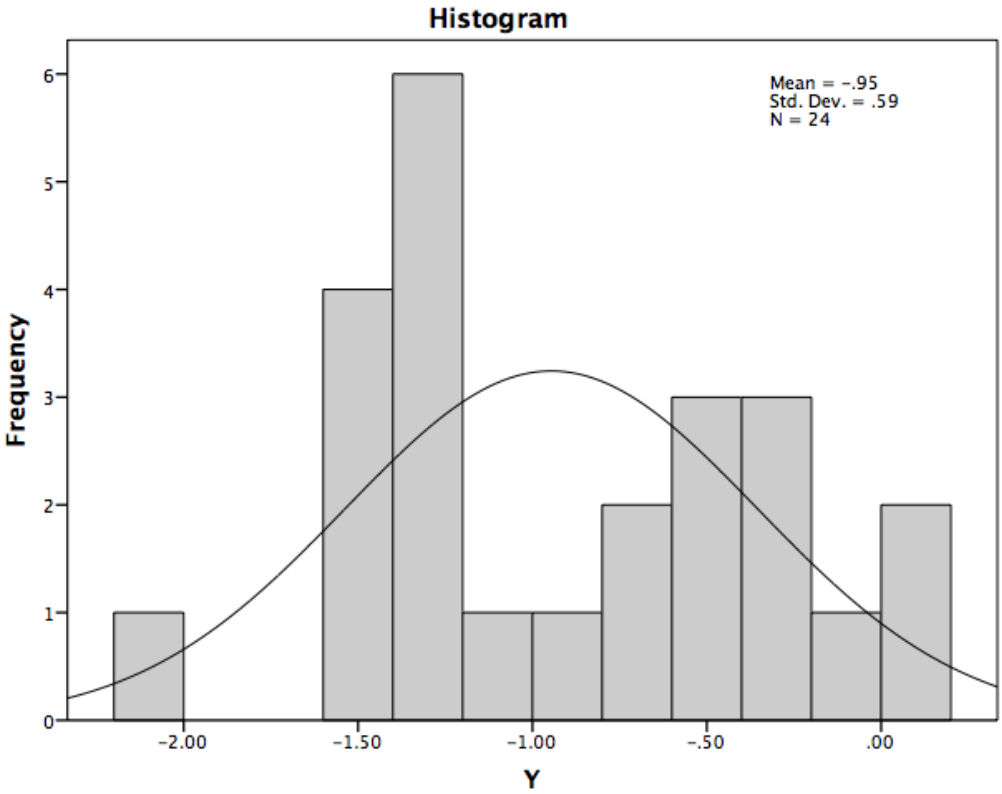
FORMULA:

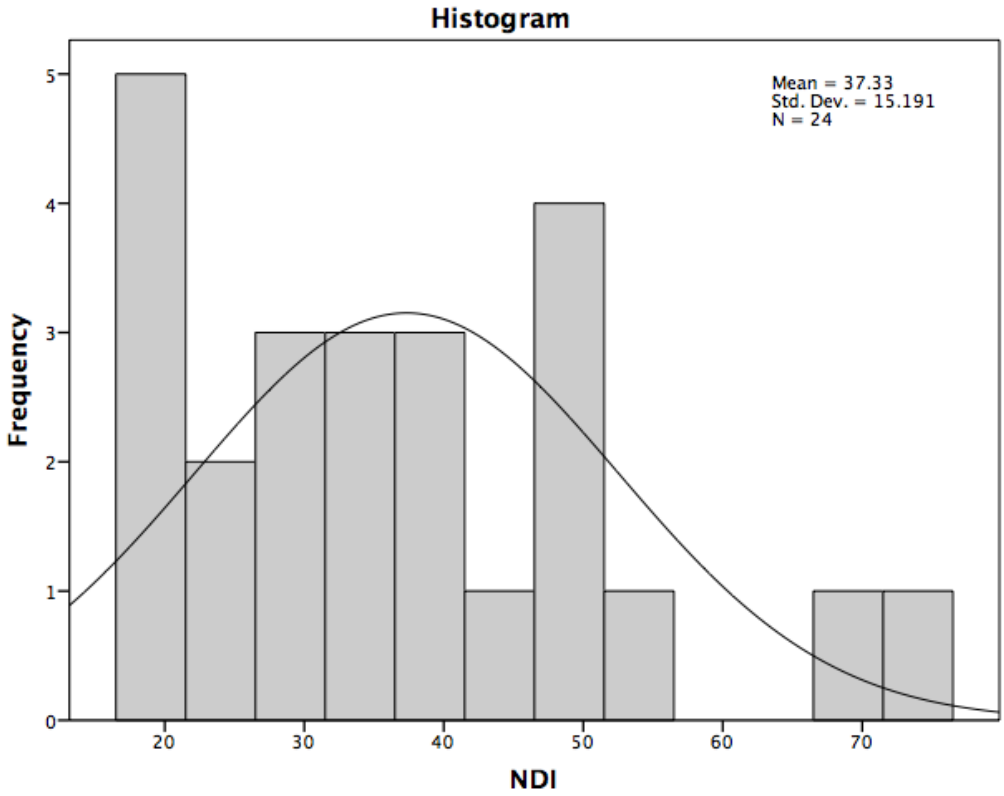
$$\frac{\text{Punteggio totale}}{\#\text{ di sezioni riempite X 5}} \times 100 = \_\_\_\_\_ \text{ Disabilità}$$

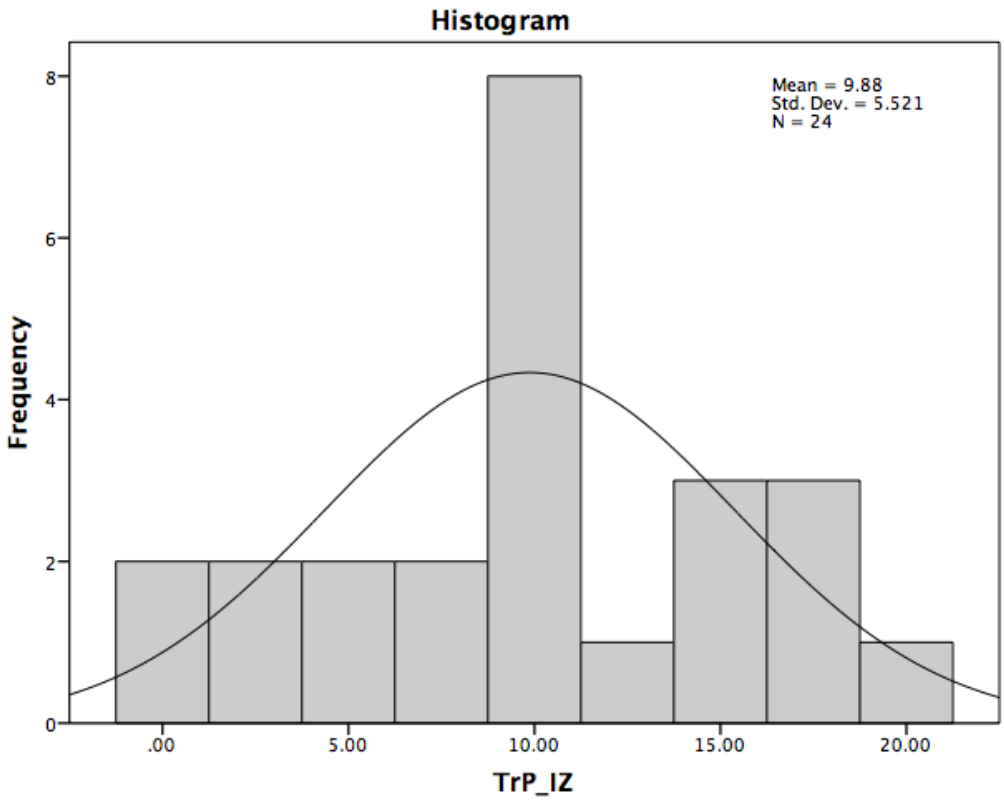
ESEMPIO:
Se 9 delle 10 sezioni sono state riempite, dividere il punteggio totale per 9 X 5 = 45.
Punteggio totale: 22
Numero delle sezioni riempite: 9 (9X5=45)
→ 22/45X100=48% di disabilità

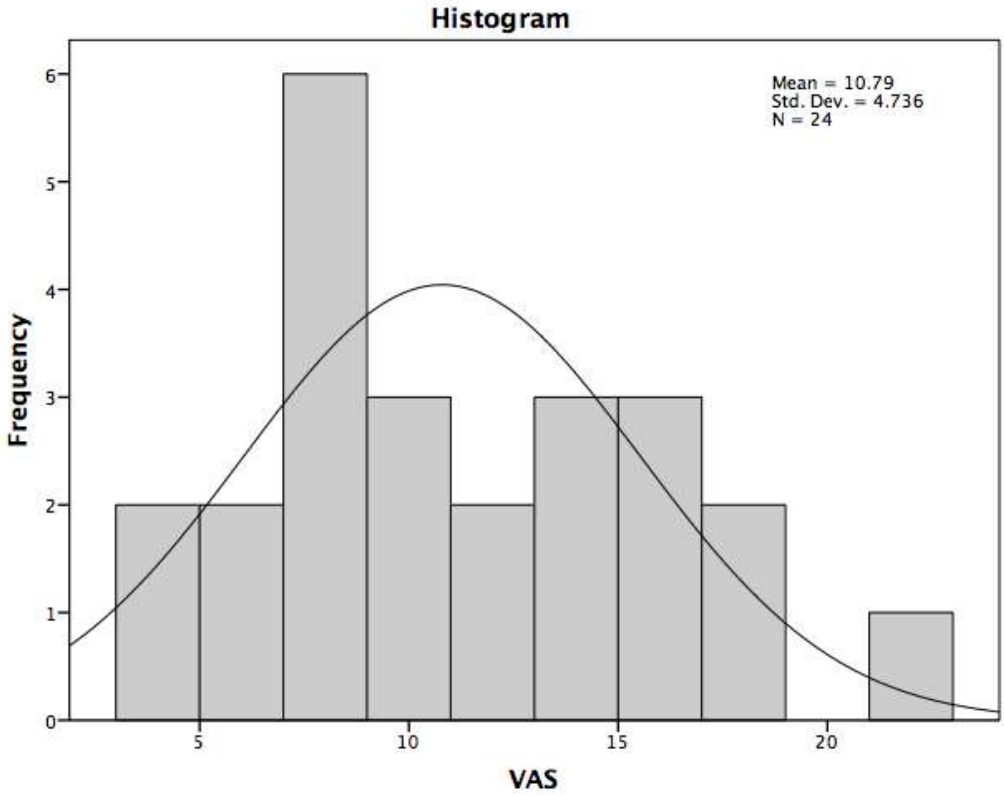
Appendix XVI: Histograms and results of the normality tests in Chapter 5.






Histogram
Mean = 37.33
Std. Dev. = 15.191
N = 24
Frequency
NDI


Histogram
Mean = 9.88
Std. Dev. = 5.521
N = 24
Frequency
TrP_IZ

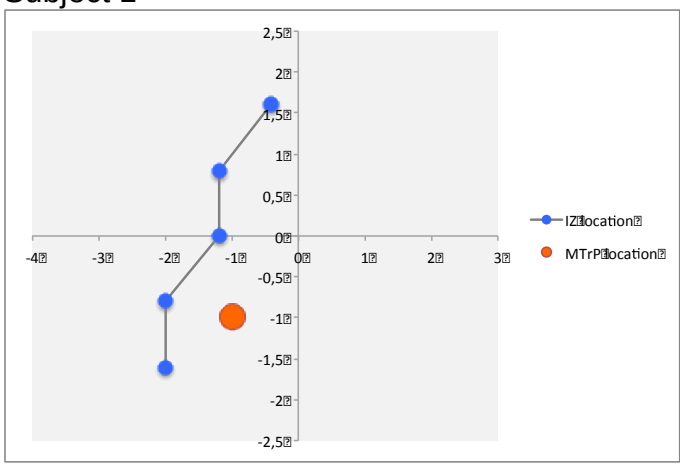


Test for normality.

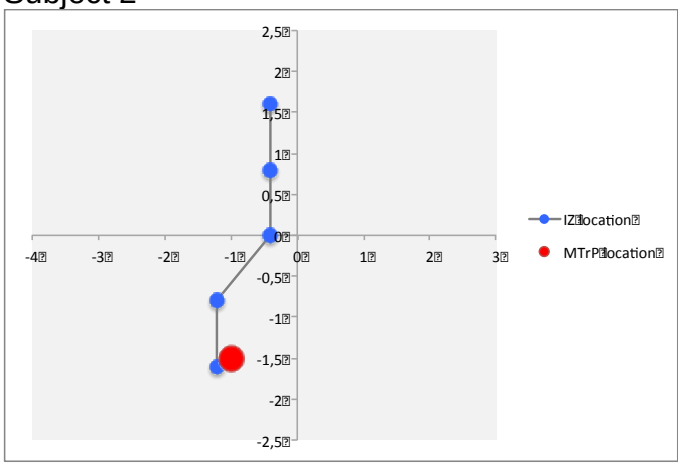
	Shapiro-Wilk		
	Statistic	df	Sig.
X	.992	24	.999
Y	.932	24	.107
TrP_IZ	.971	24	.682
PTT	.951	24	.287
NDI	.924	24	.070
VAS	.951	24	.280

Appendix XVII: IZ' and MTrP' location with respect to the ARS for each subject.

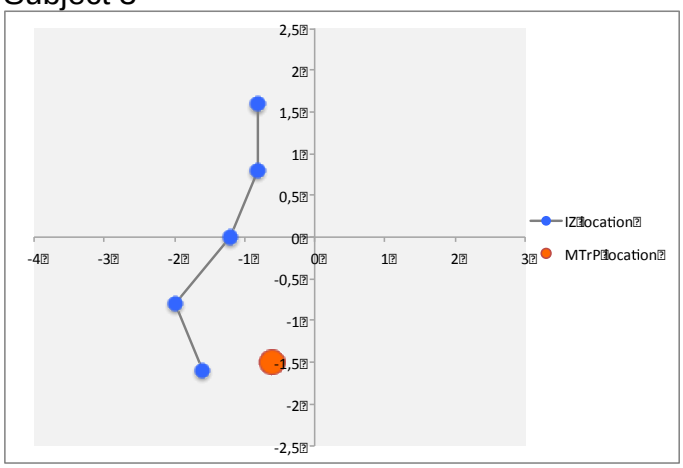
Subject 1



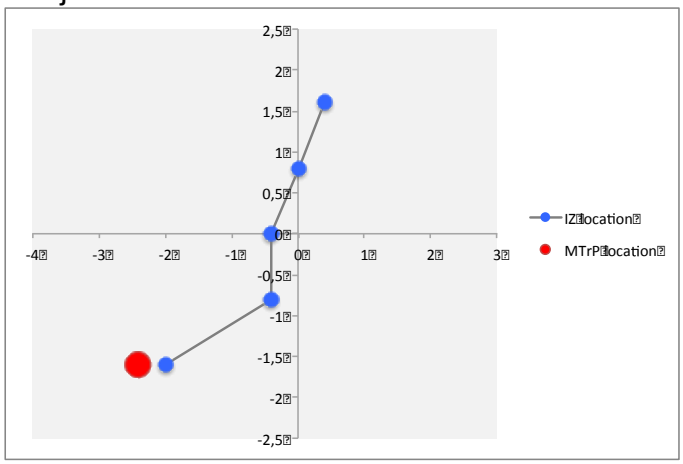
Subject 2



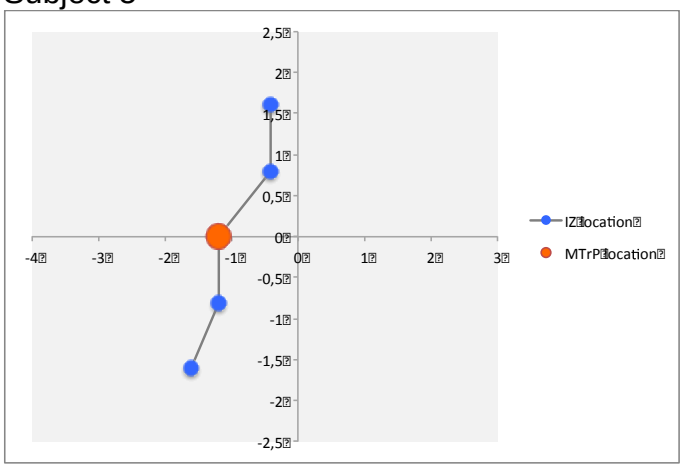
Subject 3



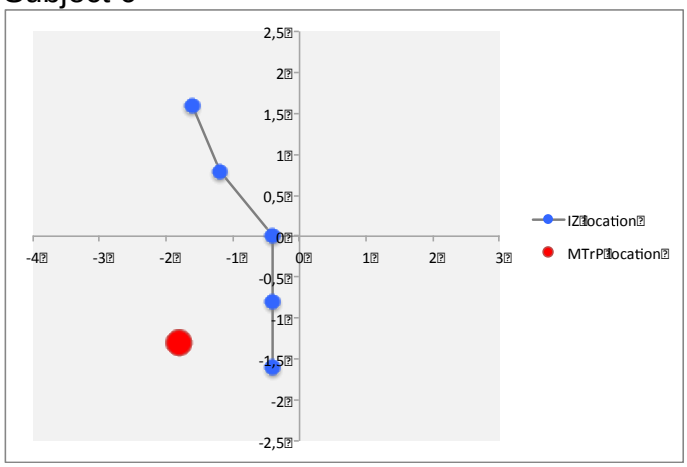
Subject 4



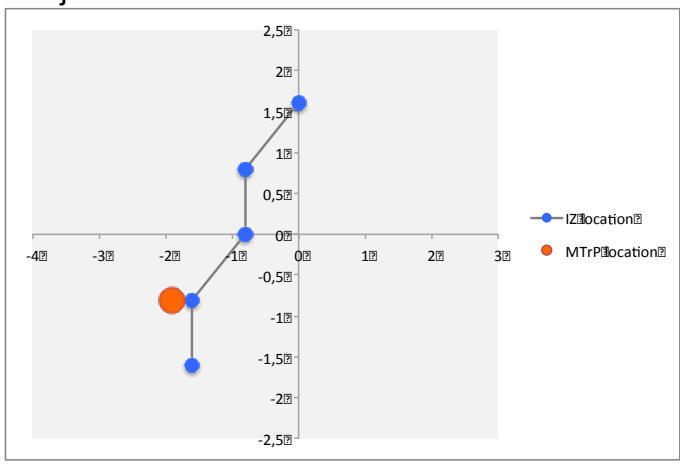
Subject 5



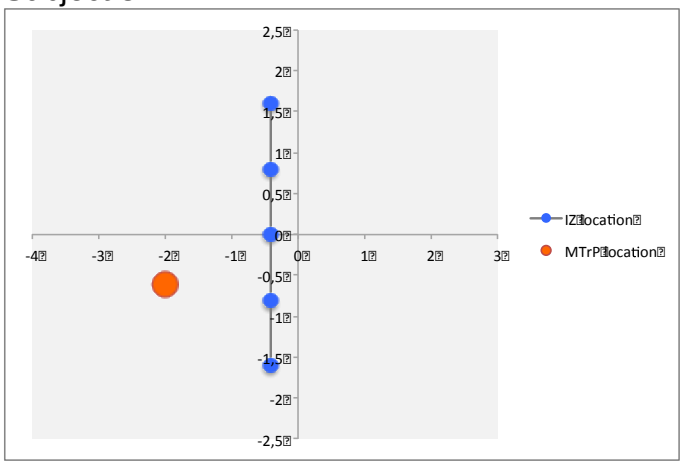
Subject 6



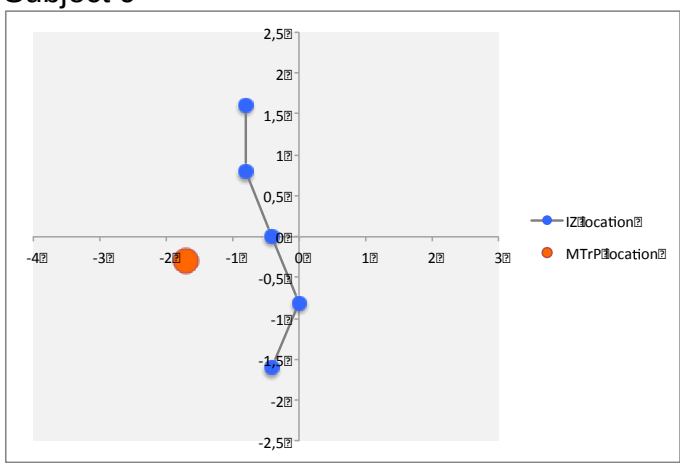
Subject 7



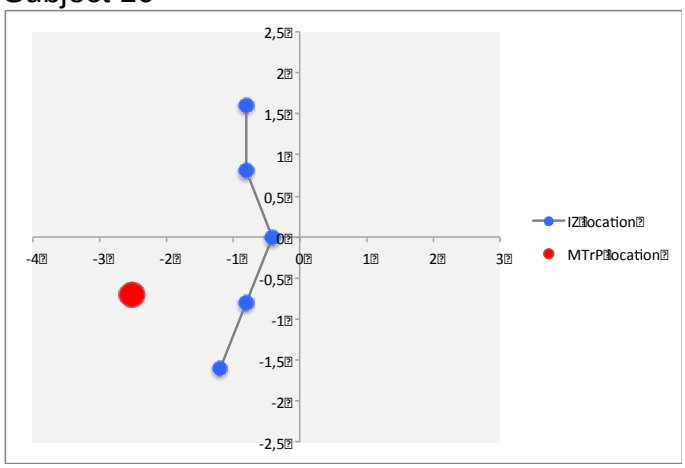
Subject 8



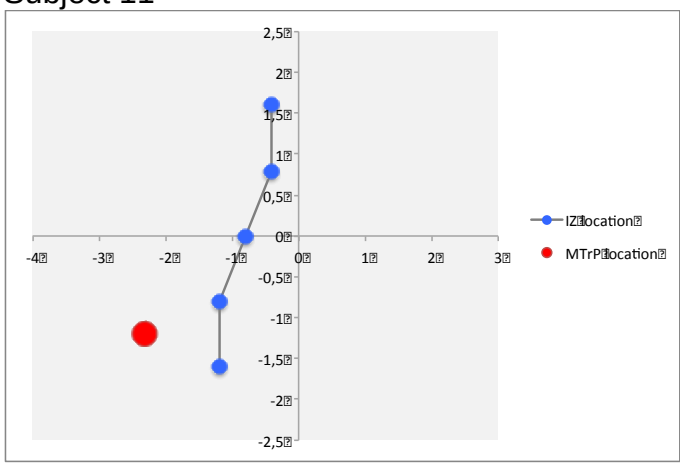
Subject 9



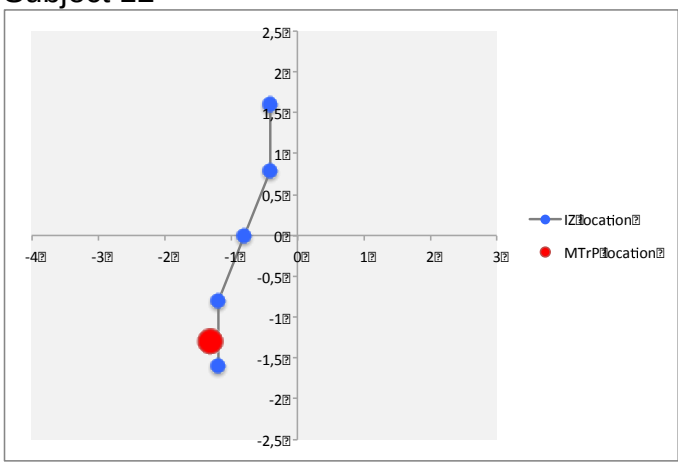
Subject 10



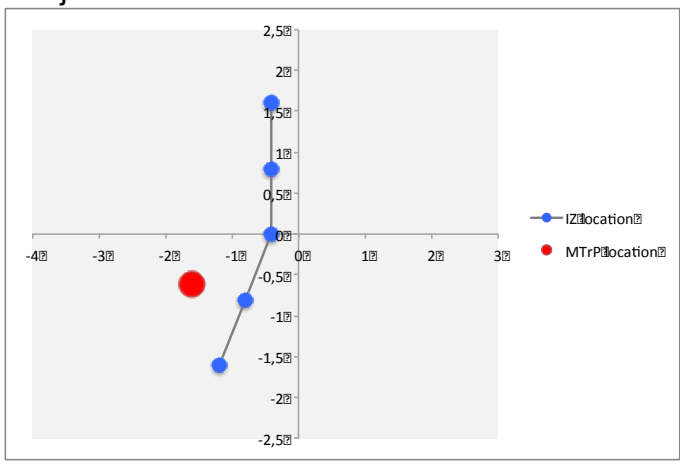
Subject 11



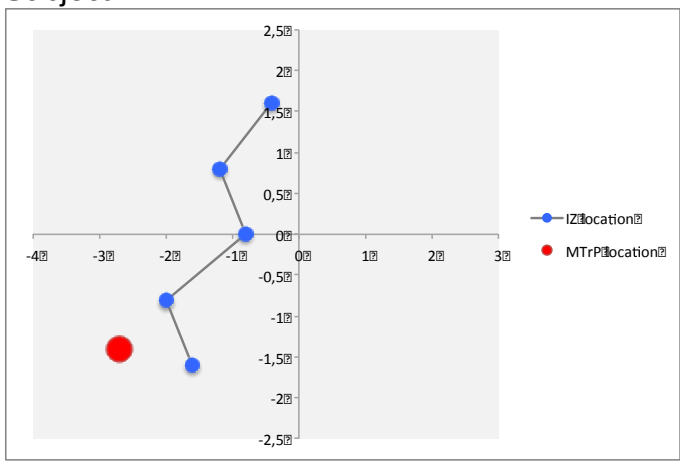
Subject 12



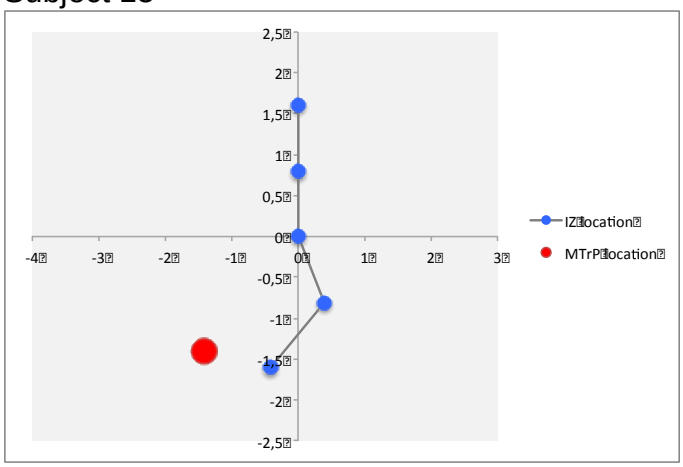
Subject 13



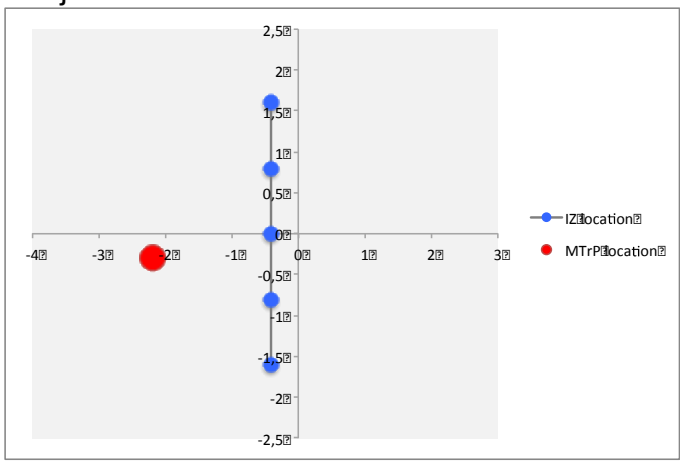
Subject 14



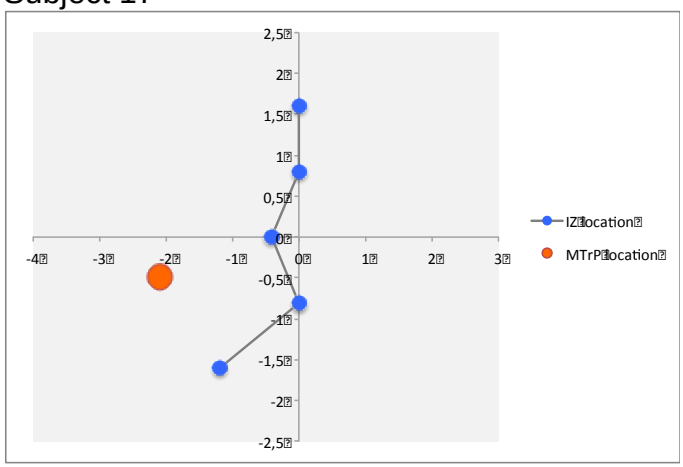
Subject 15



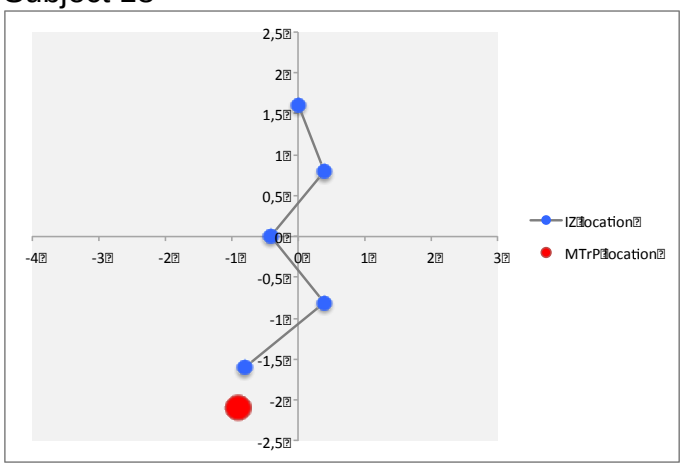
Subject 16



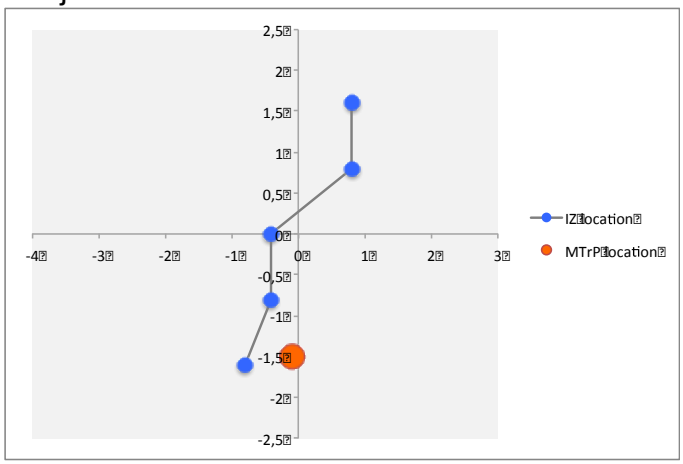
Subject 17



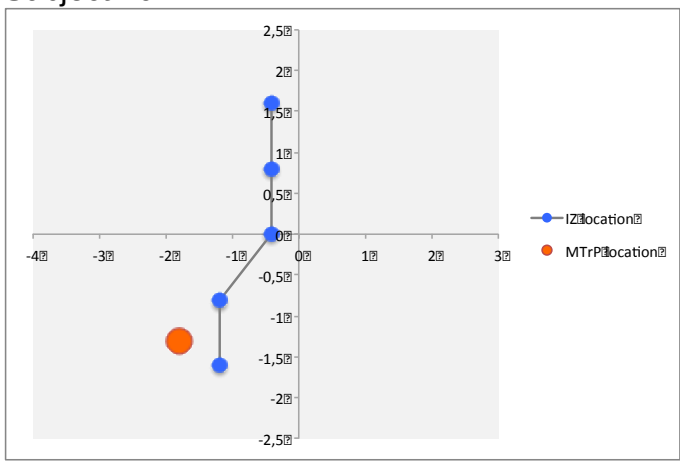
Subject 18



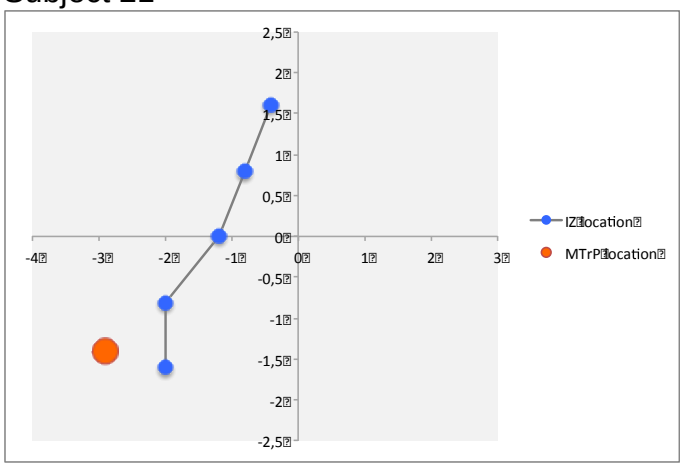
Subject 19



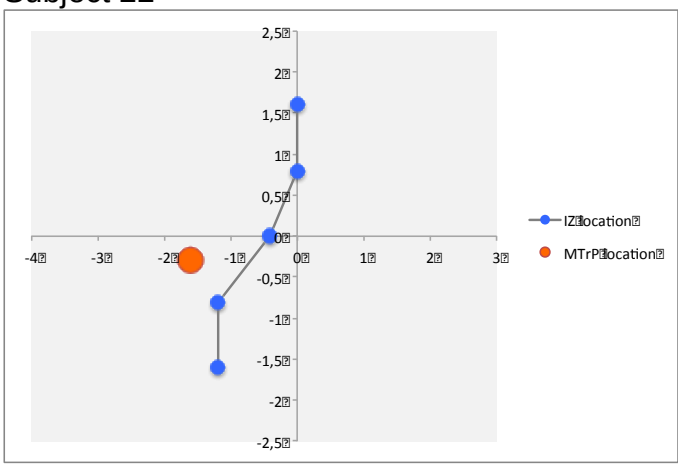
Subject 20



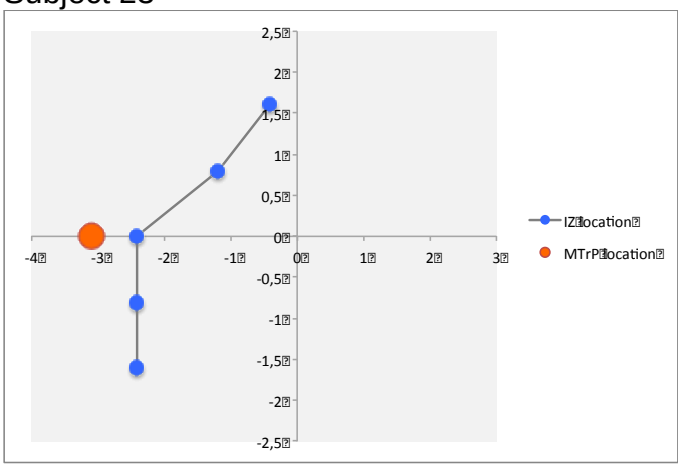
Subject 21



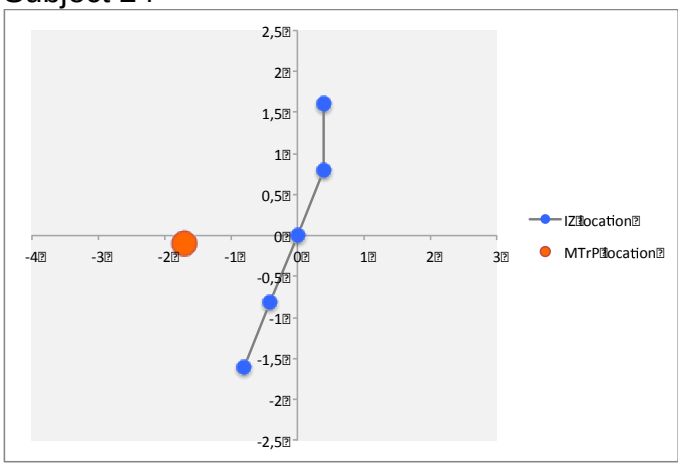
Subject 22



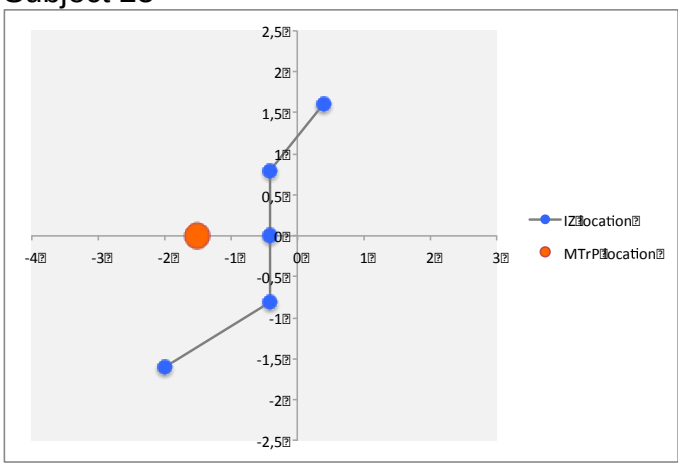
Subject 23



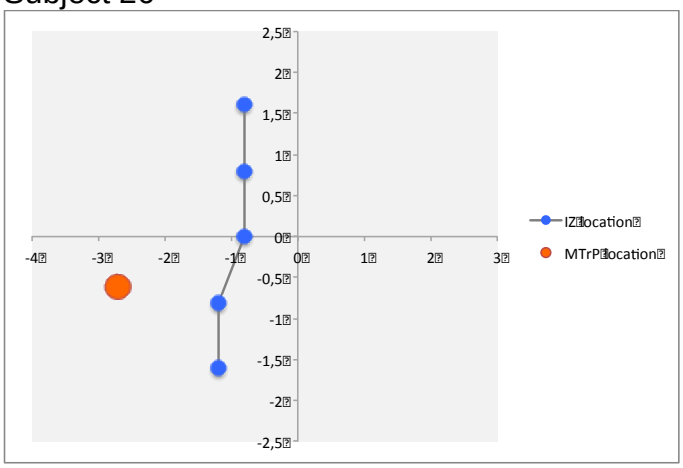
Subject 24



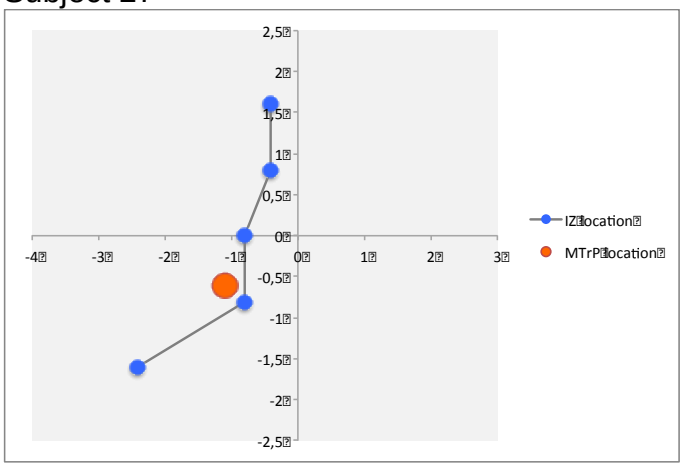
Subject 25



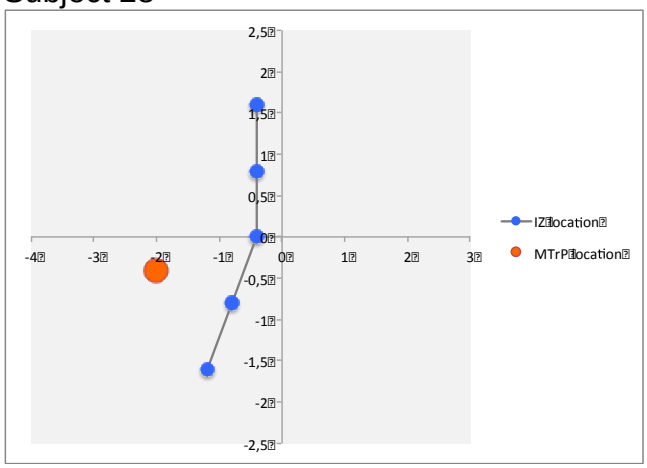
Subject 26



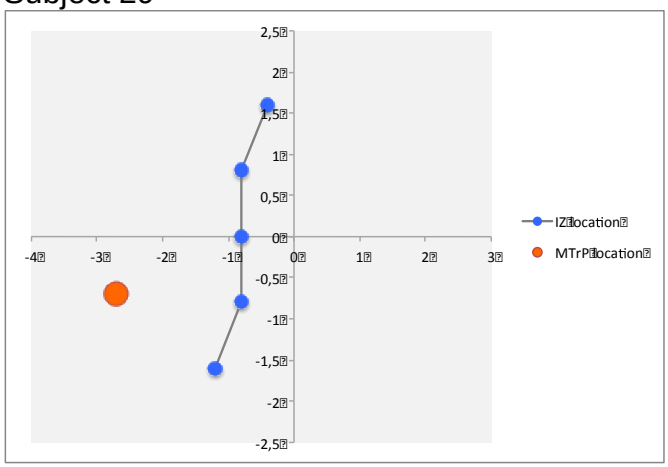
Subject 27



Subject 28



Subject 29



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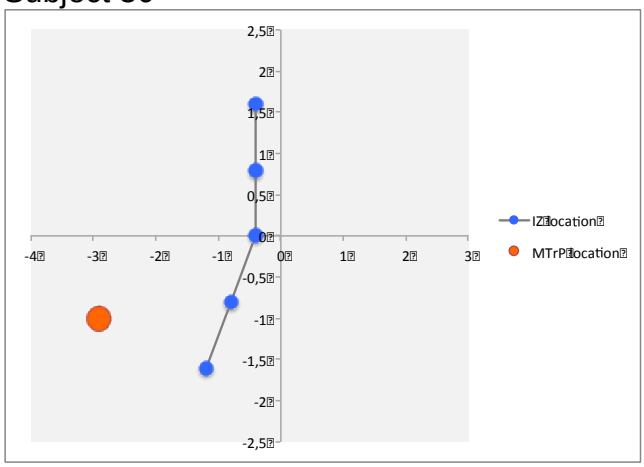

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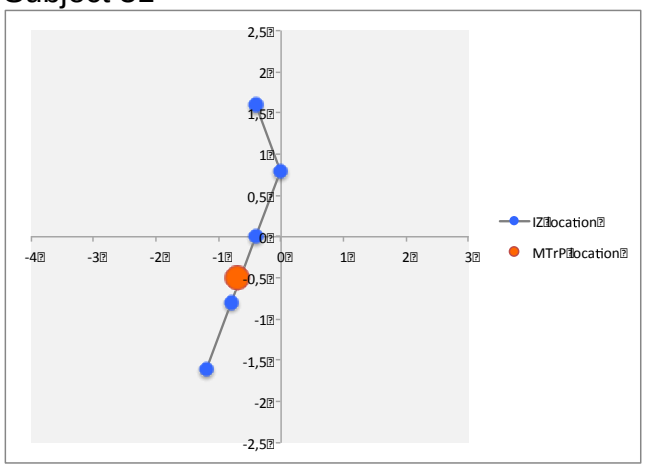

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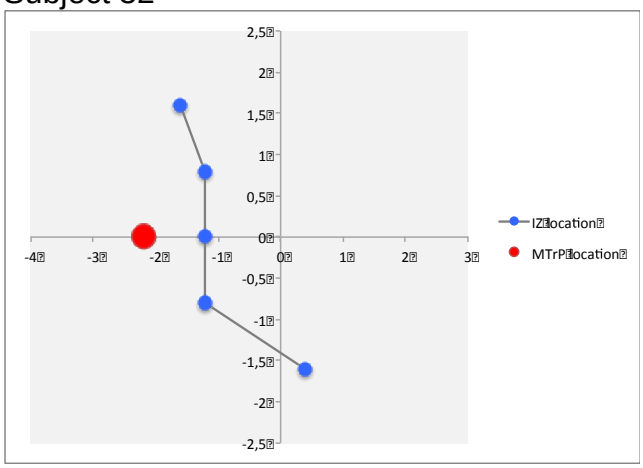

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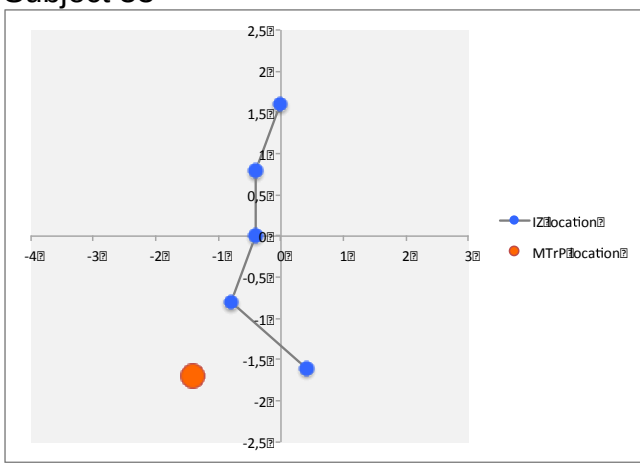

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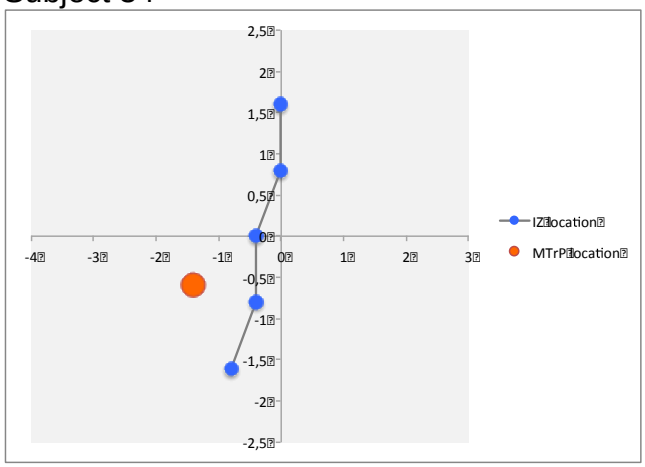

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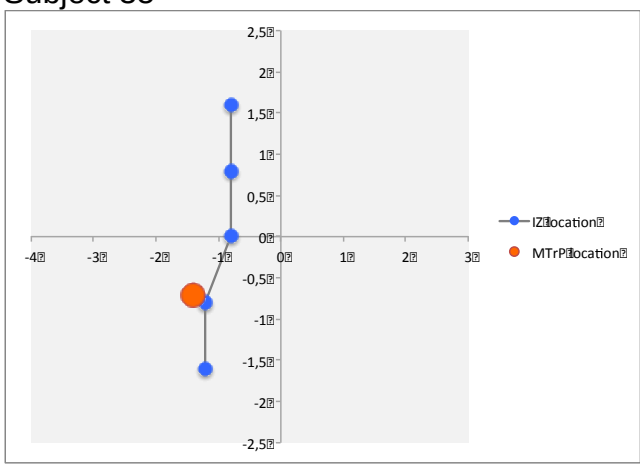

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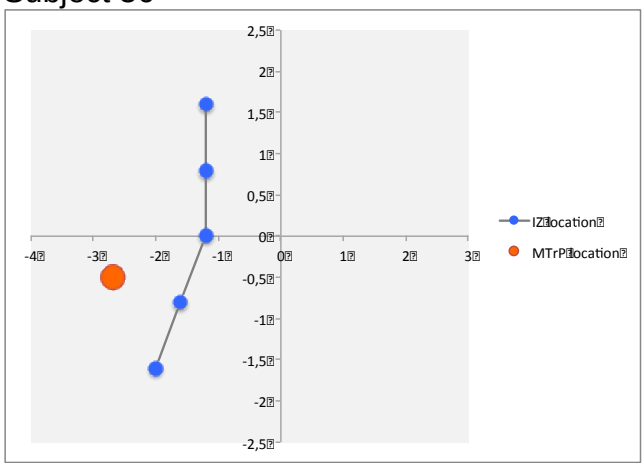

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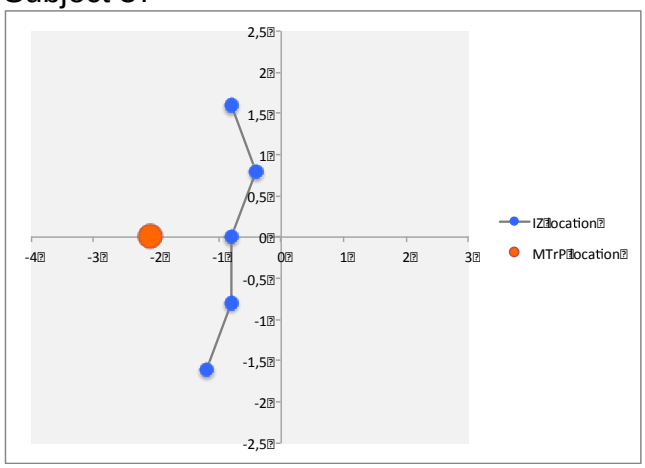

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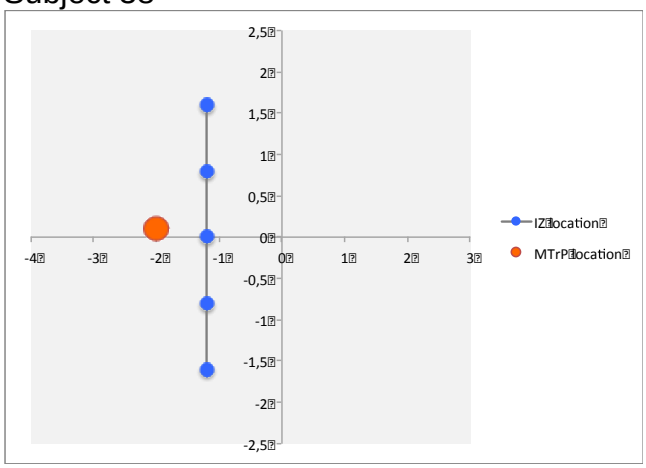

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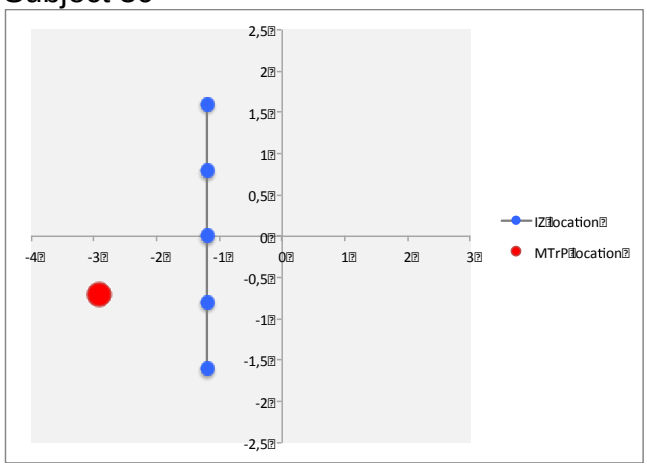

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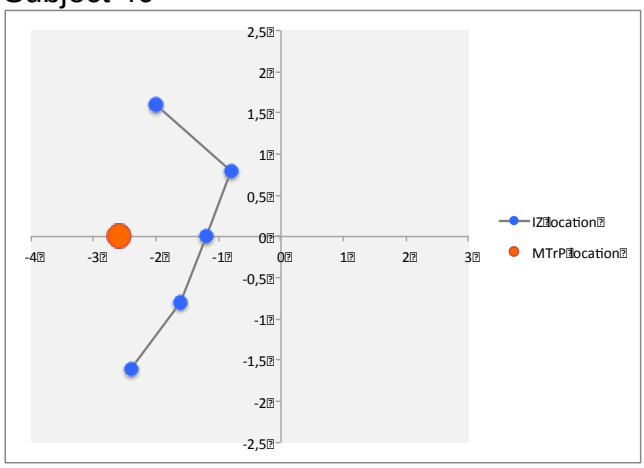

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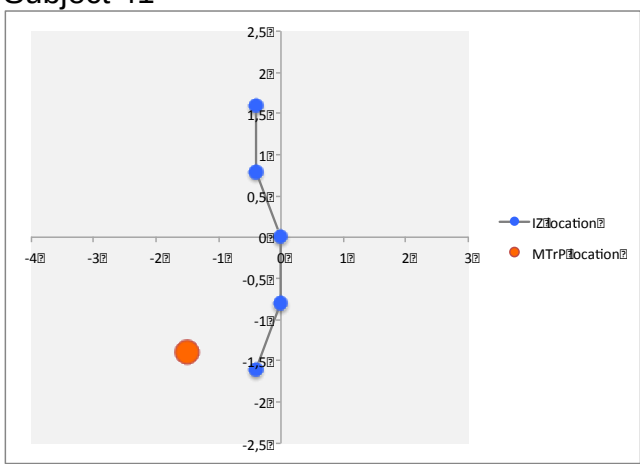

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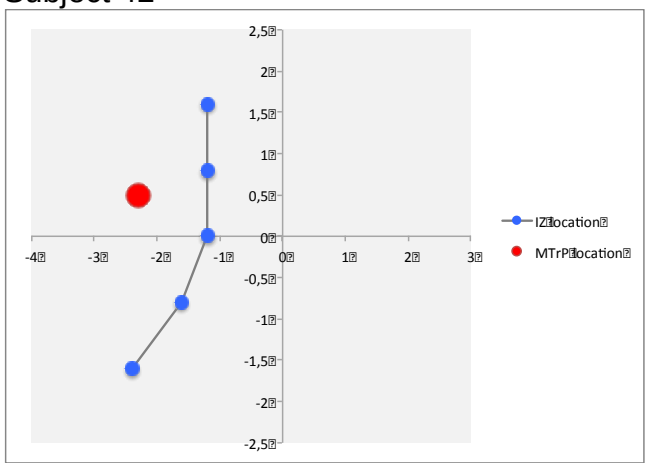

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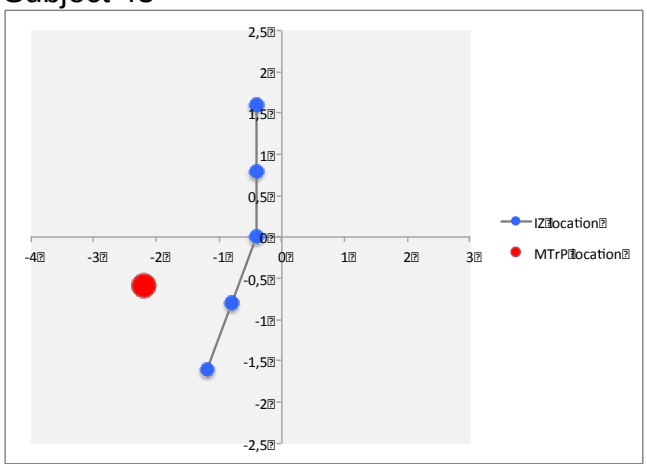

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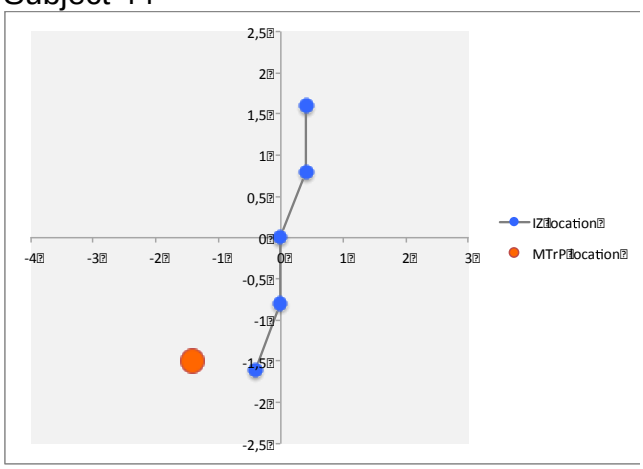

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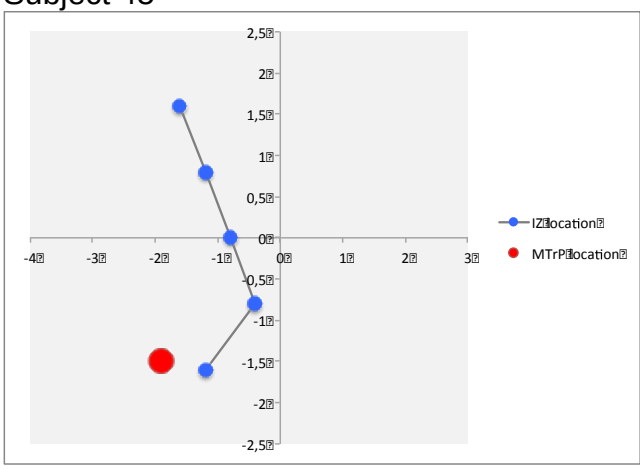

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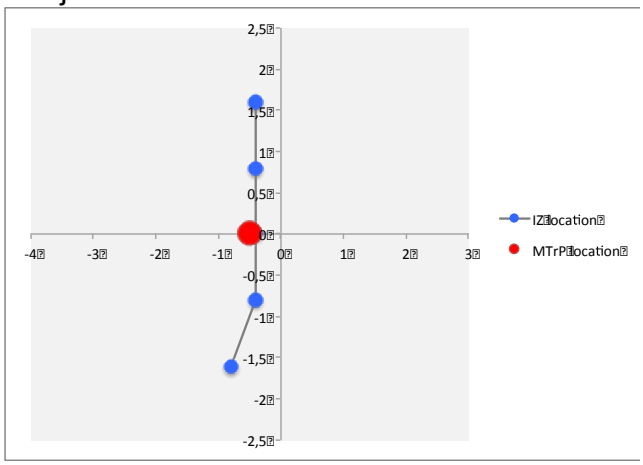

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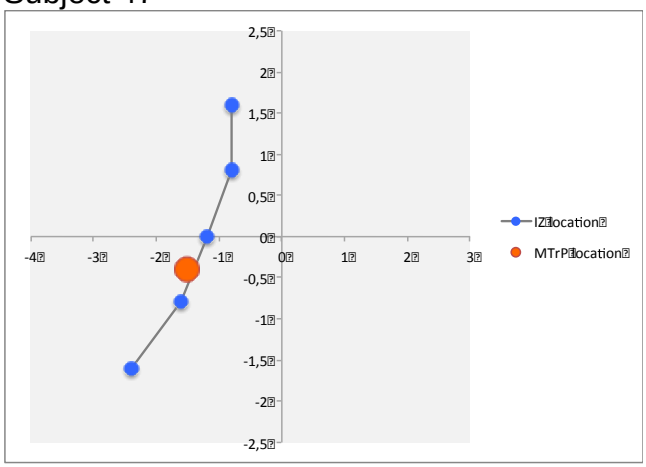

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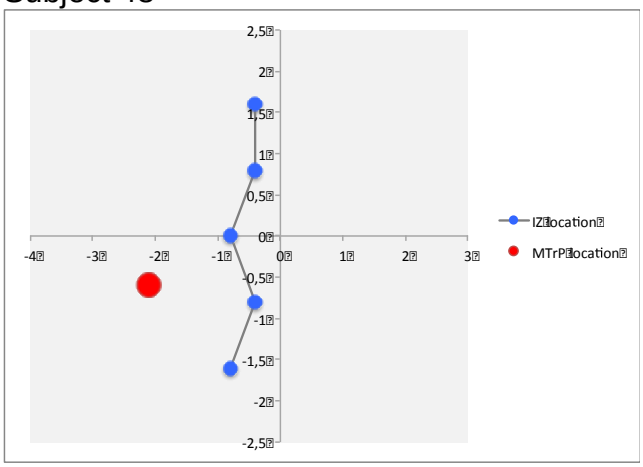

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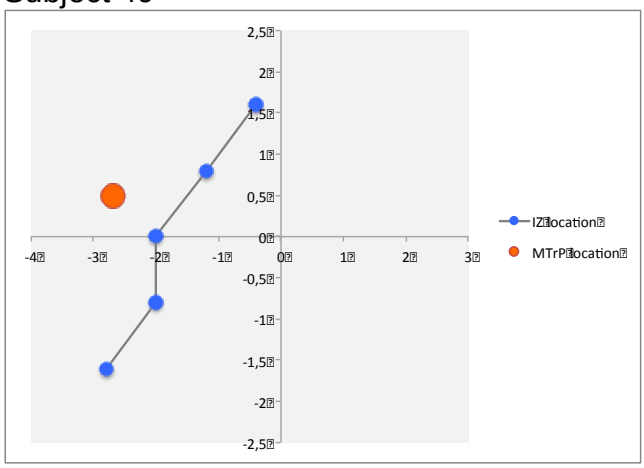

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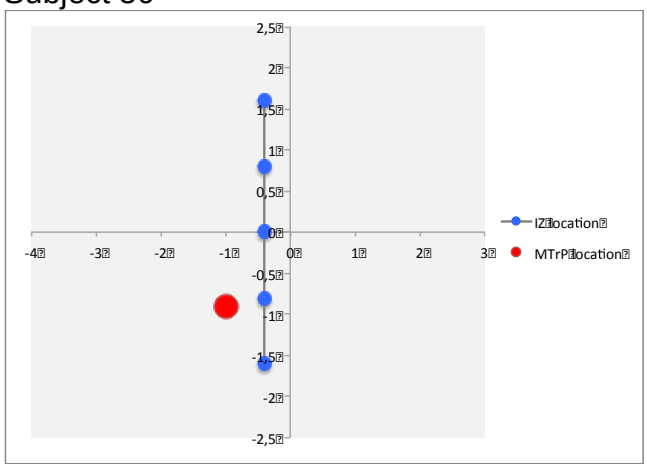

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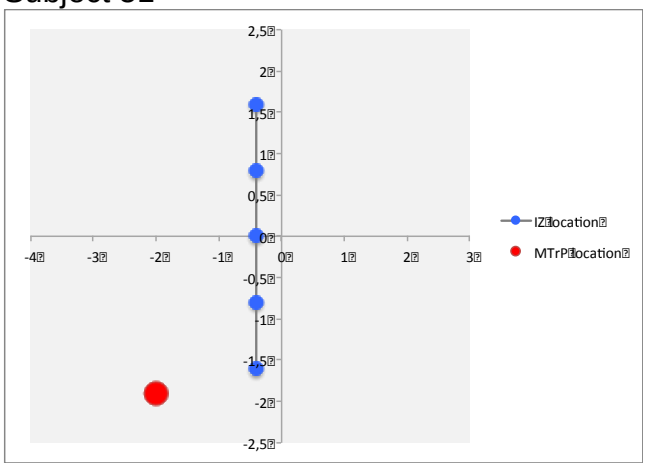

Subject 52